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ARS Conference Report on Natural Products for Control of Agricultural Pests and 5-Year Research Action Plan

Athens, Georgia
October 19-20, 1993

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Preface

An urgent need exists for biologically-active, biodegradable, naturally-derived products and other biologically based alternatives to protect pre- and post-harvest commodities from arthropod, parasite and pathogen, and weed pests. During the past few decades the consumer public has insisted on the development of alternative pest control methods to supplement and offset extensive reliance on synthetic organic chemical pesticides. Continued concerns with pesticide residues in food and ground water, and pesticide effects on non-target life forms, combined with more stringent regulation or loss of pesticides (insecticides, herbicides, and fungicides) will result in a greater reliance on environmentally sound, economical, and publicly acceptable pest control strategies. In fact, USDA has set a goal for the implementation of biologically-based Integrated Pest Management (IPM) programs on 75% of the nation's acreage by the end of this century.

Naturally-derived pesticidal products are considered to be important components of alternative control tactics. Development of environmentally-sound pest control methodology is a high priority for ARS, and is envisioned as a desired cornerstone of IPM and area-wide pest management systems for the future. If such alternatives are not developed to replace more hazardous chemicals used in agriculture, and if the public and political leaders continue to push for the elimination of the use of chemical herbicides and pesticides, the United States and other nations may inadvertently face a severe crisis in agriculture.

This conference report and 5-Year Research Action Plan for development of natural products to control agricultural pests helps to facilitate a cooperative and cohesive team effort to develop technologies for solving serious national pest problems. This has required the formulation of a comprehensive action program for the natural product area that clearly defines and states program goals and objectives, identifies each project's relevance and role, identifies activities to reach the objectives, establishes time frames needed to reach objectives, and provides a basis for participation of the scientists in planning the program. This comprehensive plan can provide (a) focus and programmatic stability, (b) a basis for monitoring and evaluating program progress, (c) a basis for developing budget estimates and allocating resources, and (d) development of team players and teamwork.

The 5-Year Research Action Plan is designed to be dynamic and responsive to needs and priorities. Progress in reaching goals will be reviewed on a periodic basis. As the program progresses, participants will play a significant role in redefining essential activities, in eliminating some proposed activities that may

result from the inherent uncertainties of research, and in assigning appropriate remaining activities or selecting new activities to achieve goals.

We express our gratitude and appreciation to all working conference speakers, discussion leaders, panelists, and attendees for participating in the organization and proceedings of the conference and in helping to formulate the comprehensive research action plan. Special appreciation is accorded to the members of the workshop Steering Committee, Don L. Bull, Wendell E. Burkholder, Stephen O. Duke, Ivan W. Kirk, James C. Locke, Richard G. Powell, Charles L. Wilson and their staffs for the considerable time and effort that they devoted from the inception of this important activity.

Robert M. Faust
National Program Leader
Fundamental & Molecular Entomology

Horace G. Cutler
Chairperson,
Steering Committee

Executive Summary

A major high priority of the Agricultural Research Service (ARS) is the development of environmentally compatible methodology for controlling agricultural pests. Natural products have enjoyed increasing attention as feasible tools for this purpose, especially as important components of integrated and area-wide pest management systems.

An ARS-wide working conference devoted to natural products for control of agricultural pests was held October 19-20, 1993, in Athens, GA. The overall purpose of the conference was to determine research priorities and major areas for emphasis with reasonable time frames to reach goals and to develop a nationally coordinated research action plan that would help unify the program into a team effort. Participants included representatives from the ARS National Program Staff (NPS), line-management, and ARS research units, as well as private industry, universities and other Federal agencies.

The ARS 5-year action research plan on natural products for control of agricultural pests is divided into five main sections: (A) Chemical Methodologies and Bioassays for Natural Products; (B) Natural Products for Arthropod Control; (C) Natural products for Parasite and Pathogen Control; (D) Natural Products for Weed Control; and (E) Delivery Systems/Application Technology for Natural Products. Also included are survey reports from ARS Management Units that detail each scientist's research accomplishments, research objectives, current and future cooperators, potential uses of research findings, research needs, and technology transfer and end-use strategies and opportunities.

A number of high-priority program needs and opportunities were identified and included: (1) establishment of close coordination between the five major research areas of the action plan; (2) establishment of a database for natural products that would include chemical names, structures, bioassay results, biodegradable properties, application technology activities, names of pesticides "lost" due to re-registration requirements, and natural product scientists active in the research areas; (3) recognition of the critical importance of bioassays as an integral part of the program; (4) establishment of teams for the re-isolation of biologically-active natural products in bulk quantity; (5) development of methodologies for fermentation, bioassay, formulation and application technology, and the use of genetic engineering, (6) establishment of specific location for major analytical equipment; (7) establishment of critical masses of researchers for each research area as resources can be made available; and (8) development of closer cooperative and non-competitive linkages with industry.

Objectives, Charge to the Workshop, and Format

The overall charge of the working conference was to assess needs and opportunities for research on natural products to control agricultural pests in terms of their potential use as environmentally compatible and economical tools for pest management. Participants were also charged with developing a nationally coordinated research action plan that would permit follow-through activities. This plan would allow ARS to form an integrated team of researchers focused on natural product research for pest control from the discovery phase to field application. The working conference was specifically designed to provide a forum for expressing views and ideas for the future.

Specific objectives were to:

(1) Foster improved communication and cooperation among ARS scientists and others having an interest in natural products for control of agricultural pests.

(2) Determine the current research status and progress, major contribution, and scientific and technological objectives of natural product research.

(3) Identify research gaps and any bottlenecks to the development and utilization of natural products as components of pest management tools.

(4) Determine research priorities and major areas for emphasis with reasonable time frames to reach goals and transfer of developed technology.

The workshop agenda is included in Appendix A. The format of the workshop was to have keynote speakers review major issues affecting ARS's efforts at developing and implementing natural product pest control strategies. Plenary panel discussions were then held to review the research focus for each of the five main subject areas, summarize activities and accomplishments currently under way in ARS, and review key priorities and other issues. ARS scientist research summaries were distributed and shared with the participants (see Appendix C).

Following the plenary meeting, individual focus groups were convened on each of the five main topics of the workshop. Each focus group developed lists of key issues and research priorities affecting the development of technologies in the respective areas. The groups also developed a proposed 5-year action plan that was then revised and approved by NPL following the meeting.

The following sections included summaries of the discussion groups' research priorities and the 5-year action plans for each main research focus area.

Introduction

The Agricultural Research Service (ARS) is the Department's principal intramural research agency. It has long-standing working relationships with the other research agencies in the Department, the State Agricultural Experiment Stations, and the private research sector. The ARS also works closely with the action agencies in the Department and serves as the research arm for many of them. Supported by appropriated funds from Congress, ARS provides the ability to perform long-term, high-risk research, ability to respond to changing technical goals, an organizational structure ensuring research program accountability and coordination; ability to focus research on gaps in knowledge that are barriers to problem solutions, and capability to form, disband, or coordinate interdisciplinary or multilocation research teams from a large, diverse scientific workforce of research scientists, including postdoctoral research associates.

The overall mission of the ARS is to develop new knowledge and technology needed to solve technical agricultural problems of broad scope and high national priority in order to ensure adequate production of high-quality food and agricultural products to meet the nutritional needs of the American consumer, to sustain a viable food and agricultural economy, and to maintain a quality environment and natural resource base.

Although there are a number of major issues important to the ARS research mission, such issues as pesticide residues, food safety, air and water pollution, and other environmentally related impacts from agriculture have become increasingly critical. In terms of pesticide residues the consumer public has insisted on development of safer pest control methods to supplement and offset extensive reliance on synthetic organic chemical pesticides. Progress has been made in such areas as host-plant resistance, traditional biological control and other biologically-based tactics, cultural control and pest management systems. Although good progress has been made in these areas, synthetic chemical pesticides are still our major means of protecting the nation's food and fiber crops from pests. New, environmentally-compatible pest control technologies are slowly replacing synthetic pesticides. This will be an even more critical issue as more registered pesticides are banned.

Increased research on the discovery and development of natural products for control of agricultural pests is the focus of this report and includes research approaches which are detailed in a comprehensive action plan that targets arthropod, pathogen and nematode, and weed pests. Consideration is given to chemical methodologies and bioassays which are important to natural product development, as well as delivery systems and application technology. The overall goal of the research program will be to

discover and develop new groups of biosafe natural pest control products. These would include attractants, repellents, antifeedants, phagostimulants, oviposition stimulants, toxicants, growth regulators, fungicides, nematocides, bacteriocides, phytotoxins and other biologically-active materials. The work will encompass the identification, isolation, and characterization of such materials; it will by necessity include screening and bioassay, extraction, synthesis, defining mode of actions, formulation, and evaluation in the laboratory and field for efficacy and safety, and ultimately testing in area-wide management and IPM programs. As mentioned, application technology and the development of delivery systems will be an important component of the effort as well as the use of genetic engineering technology to develop pest tolerance. New groups of agents having novel modes of action are especially desirable and should be of such a nature that the target pest could neither detoxify nor develop resistance to them very readily.

High Priority Needs and Panel Recommendations

Each research area viewed its needs and recommendations from a slightly different perspective and this will be seen upon examining the panel recommendations. Discussions in open forum at the close of the workshop gave further insight into needs. All pertinent comments were added to the recommendations from each research area and condensed to give the top 11 high priority needs.

- Continue to develop natural products as alternatives to "hard" chemical pesticides.
- Continue to identify biologically-active natural compounds from plants, microorganisms, and other promising sources.
- Establish close coordination and internal cooperation between the five major research areas of the action plan.
- Schedule meetings at regular intervals for the various teams active in natural product research.
- Establish a database for natural products to include: chemical names, structures, bioassay results, biodegradable properties, application technology activities, names of pesticides "lost" due to re-registration requirements, and natural product scientists active in the research areas.
- Recognize the critical importance of bioassays as an integral part of the program.
- Establish teams for the re-isolation of biologically active natural products in bulk quantity.
- Develop methodologies for fermentation, bioassays, formulation and application technology, and use of genetic engineering.
- Strive to establish critical masses of researchers in a centralized location for each research area as resources can be made available.
- Establish specific locations for major analytical equipment and provide the necessary technical support. (e.g. mass spec.; FAB MS; ^{1}H and ^{13}C NMR; X-ray crystallography).
- Develop closer cooperative linkages with industry and ascertain clear-cut industry needs in terms of useful products in a manner that is complimentary and not competitive.

The individual discussion groups' priority research needs are listed as follows:

PANEL A
(Chemical Methodologies and Bioassays)
Needs and Recommendations

A. Chemical Methodologies

- Co-ordinate and catalog the chemical methodologies available in the agency.
- Continue to identify locations at which specific pieces of technology are available. For example, FAB-MS; ^{1}H and ^{13}C NMR; High resolution MS; x-ray crystallography; super-critical-fluid extraction, etc.
- Link the availability of the above mentioned equipment with specific CRIS's.
- Develop and maintain a database of researchers who have special skills relative to chemical methodologies.
- Centralize equipment needs on a practical basis: realizing that all the components under one roof is ideal but impractical.

B. Bioassays

- Formulate a database of bioassay capabilities within the agency to serve as a reference for scientists.
- Spell out bioassays presently used with specific CRIS's.
- Emphasize that bioassay systems should serve the needs of individual research programs.

C. Linkages and Cooperation

- Increase internal cooperation. Foster links between laboratories that can furnish equipment, or bioassay needs.
- Recognition that interpretation of data (nuclear magnetic resonance) is an essential part of the research package.
- Recognition that bioassays are an essential ingredient in any successful natural products program.
- Reiterate that ARS should not compete with industry but should form a vital cooperative component.

PANEL B
(Natural Products for Arthropod Control)
Needs and Recommendations

A. Expertise and Concomitant Needs

- Increase cooperation between natural products chemists, molecular biologists, and formulation/application technology specialists by joint meetings/newsletters.
- Whenever feasible locate biological/chemical/engineering experts in close proximity.

B. Communication Needs

- Foster communication/collaboration among scientists.
- Construct an improved database of research, researchers, and research skills, building on RMIS information.
- Disseminate the list of lost pesticides (due to re-registration) so that specific natural product substitutes can be found. Match the needs of agrochemicals with the research.
- Make early contact with application technologists to facilitate early practical use of natural products.

C. New Leads

- Reexamination of previously identified biologically active natural products for new uses.
- Evaluate new "chemotypes", or structurally related analogs, for new leads.
- Increase research on macromolecular natural products that exhibit biological activity.
- Increase research on toxicology to provide new targets in biochemical pathways.
- Investigate subsistence/traditional agricultural practices for useful leads.
- Investigate natural products at the whole organism level for effects on orientation, movement, etc.

D. Ecological Considerations

- Establish more rigorous field testing, with special concern for impact on natural enemies.
- Critically evaluate natural products to determine the context in which they affect arthropods so as to identify application technology needs (formulation, timing, concentration, and placement).

E. Funding, Support, and Organization

- Examine the need for more support and higher priority for natural product research.

PANEL C
(Natural Products for Parasite and Pathogen Control)
Needs and Recommendations

A. Establish and improve the development of new, safe and economical alternatives to currently-used pesticides which may not be re-registered.

- Plan alternative fumigants (methyl bromide replacement)
- Plan new natural pesticides for preharvest disease control.
- Plan new natural pesticides for postharvest disease control.

B. Develop and improve research strategies and approaches.

- Identify, exploit, and characterize host resistance mechanisms.
- Identify, exploit, improve and develop biocontrol organisms <u>in conjunction with</u> their endogenous biologically active natural products.
- Identify natural compounds, with biological activity from plants, microorganisms, and other sources.
- Develop methodologies for:
    - Fermentation
    - Bioassays
    - Application
    - Formulation
    - Genetic engineering

C. Examine and elucidate the mode of action of biologically active natural products so that chemical analogs to block specific chemical pathways can be found for practical pesticide development.

PANEL D
(Natural Products for Weed Control)
Needs and Recommendations

A. Formulate better linkages within and between disciplines.

- Nominate NPS/Coordinators to facilitate and coordinate natural products research and development
- Identify which companies need what products (weed control agents, fungicides, growth regulators, etc.).
- Conduct an annual research planning meeting for natural products.

B. Need critical mass in one location.

- In order to create good linkages.
- To fully exploit natural product research and development.

C. Establish standard bioassay systems and a bioassay database.

- Use specific standard bioassay systems to successfully conduct research programs.
- Use final bioassays that are acceptable to industry.
- Use inexpensive and easily reproduced bioassays.
- Establish a database for bioassay results of both natural products and synthetic compounds.
- Establish a database of the biodegradable properties of natural products.

D. Determine the mode of action and structure activity relationship of natural products.

- To classify and categorize new herbicides.
- May involve new and different biochemical pathways.

E. Examine plants and microorganisms and other sources for new herbicides.

- Thousands of different germplasms await exploitation.
- As sources of novel compounds.

F. Form linkages with biocontrol research.

- Many biocontrol agents exert their influence by secreting natural toxins into their environment.

G. Form linkages with application technology and formulation.

- To reasonably extend and control the lifespan of biodegradable natural products.

PANEL E
(Delivery Systems/Application Technology for Natural Products)
Needs and Recommendations

A. Linkages with natural product discovery and development researchers are essential for effective research on application technology.

- Develop and maintain databases of current research and allied researchers.
- CRIS's need formulation and application technology added during preparation.
- Application technology should be included in CRADA's

B. Protocol for determining mammalian safety of natural products prior to research on application technology.

- Establish appropriate teams to develop and implement this capability.

C. Increase research emphasis on application technology by agency managers and scientists, for natural products.

- Establish earlier consideration for formulation and application technology requirements.
- Develop a mechanism to review and prioritorize natural products for further development, scale-up, and field-scale evaluation.
- Increase commitment of resources and SY's by agency for research on application technology.
- Ensure agency commitment to program continuity on application technology.

D. Sufficient quantities of consistent quality natural products to permit determination of optimum rates and identification of application problems relative to effective control and minimal environmental contamination.

- Exploit high-priority products through industry-supported CRADA's. If a CRADA is not forthcoming, the agency should provide the resources and capability for pilot-scale formulation and production to provide sufficient amounts of high priority natural products for application research.

E. Increased interaction with industry.

- Develop and maintain current database of industry representatives and new products.

Note: Panel attendance indicated 9 SY's in discovery/development to 1 SY in formulation/application. Reduction of "hard" pesticide use 75% in 7 years will be a difficult goal with these ratios.

5-YEAR ACTION PLANS
FOR SPECIFIC RESEARCH AREAS

The following tables represent the summarized 5-Year Action Plans for each of five main research areas.

RESEARCH AREA A

Chemical Methodologies and Bioassays for Natural Products

RESEARCH AREA A: CHEMICAL METHODOLOGIES AND BIOASSAYS FOR NATURAL PRODUCTS

Activity Code	Assay Method	Natural Prod.	Source	Activity During Year 1	2	3	4	5	Investigators [a]
A.1 Chemical Methods									
A. 1. a	Supercritical fluid extraction	Oil,soap,wax, other	Plant	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	JWK
A. 1. b	HPLC	Acid,ester,alcohol	Micro	Lab bioassay	Lab. bio., eval.	Eval., tech. trans.	Technology trans.	Technology trans.	RAM
A. 1. c	Countercurrent	Any	Plant, micro	Lab bioassay	Lab. bio., eval.	Eval., tech. trans.	Technology trans.	Technology trans.	All
A. 1. d	Planar chromatography	Any	Plant, micro	Lab bioassay	Lab. bio., eval.	Eval., tech. trans.	Technology trans.	Technology trans.	All
A. 1. e	Spectral analysis	Any	Plant, micro	Lab bioassay	Lab. bio., eval.	Eval., tech. trans.	Technology trans.	Technology trans.	All
A. 1. f	Databases	Any	Plant, micro	Lab bioassay	Lab. bio., eval.	Eval., tech. trans.	Technology trans.	Technology trans.	All
A. 2 Bioassays									
A. 2. a	Etiolated wheat coleoptile	Any	Bacterial, fungal	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	HGC
A. 2. b	Antibacterial	Any	Bacterial, fungal	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	HGC
A. 2. c	Antifungal	Any	Bacterial, fungal	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	HGC
A. 2. d	Antitumor	Any	Bacterial, fungal	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab bioassay, isolation, identification	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	Culture collection, identification, lab, field bioassay, isolation, identification, tech. trans.	HGC
A. 2. e	Greenhouse whitefly	Any	Tobacco	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	GWP
A. 2. f	Sweet potato whitefly	Any	Tobacco	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	Evaluation, technology trans.	GWP

RESEARCH AREA A: CHEMICAL METHODOLOGIES AND BIOASSAYS FOR NATURAL PRODUCTS

Activity Code	Assay Method	Natural Prod.	Source	Activity During Year 1	2	3	4	5	Investigators[a]
A.2 Bioassay (Cont.)									
A. 2. g	b-mannanase	N/A	N/A	Lab bioassay	Lab bioassay	Lab bioassay	Lab bioassay	Lab bioassay	RP
A. 2. h	Uronic acid oxidase	N/A	N/A	Lab bioassay	Lab bioassay	Lab bioassay	Lab bioassay	Lab bioassay	RP
A. 2. i	Duckweed cytotoxicity	Any	Fungal	Synthesis, isolation, identification	Lab bioassay, isolation, identification	Field bioassay, isolation, identification	Field bioassay, isolation, identification	Field bioassay, isolation, identification	HKA
A. 2. j	Hydroponic seedling	Any	Bacterial, fungal	To be determined					REH
A. 2. k	Insecticidal	Any	Plant	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	JKP
A. 2. l	Nematocidal	Any	Plant	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	JKP
A. 2. m	Antifungal	Any	Plant	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	JKP
A. 2.n	Allelopathic	Any	Plant	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	Isolation, identification	JKP
A. 2. o	Antifungal	Any	Micro	Isolation, identification	Isolation, identification	Technology transfer	Technology transfer		KDB
A. 2. p	Kairomone (insecticide)	Any	Plant, insect	Isolation, identification	Isolation, identification	Technology transfer	Technology transfer		BFB
A. 2. q	Allomone (insecticide)	Any	Any	Isolation, identification	Isolation, identification	Technology transfer			BFB
A. 2. r	Mexican fruit fly attractant	Any	Bacterial	Isolation, identification, lab bioassay	Isolation, identification, lab bioassay	Isolation, identification, lab bioassay	Lab bioassay		DR
A. 2. s	Avian repellent	Any	Insect	To be determined					JWN
A. 2. t	Prostaglandin synthase inhibition	Any	Insect	To be determined					JWN
A. 2. u	Antibacterial Gram +	Any	Insect	To be determined					JWN
A. 2. v	Insect cell culture cytotoxicity	Any	Plant	To be determined					RDS, AAB,CRH
A.2.w	Topical/insects	Acyl sugars	Tobacco	As needed	As needed				JWN

a) Investigators Code: See list with investigator name and location at the end of this Research Area.

INVESTIGATOR CODES
RESEARCH AREA A

CODE	NAME, LOCATION
HKA	H.K. Abbas, Stoneville, MS
AAB	A.A. Bell, College St., TX
BFB	B.F. Binder, Ames, IA
HGC	H.G. Cutler, Athens, GA
KDB	K.D. Burkhead, Peoria, IL
REH	R.E. Hoagland, Stoneville, MS
CRH	C.R. Howell, College St., TX
JWK	J.W. King, Peoria, Il.
RAM	R.A. Moreau, Philadelphia, PA
JWN	J.W. Neal,Beltsville, MD
GWP	G.W. Pittarelli, Beltsville, MD
RP	R. Pressey, Athens, GA
JKP	J.K. Peterson, Charleston, SC
DR	D. Robacker, Weslaco, TX
RDS	R.D. Stipaniovic, College St., TX

RESEARCH AREA B

Natural Products for Arthropod Control

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.1 ATTRACTANTS									
B.1.a	*Plodia interpunctella*	N/A [b]	Conspecific larvae and cracked grain	Nat. prod. isolation	Cont. nat. prod. isolation	Lab testing	Continue lab testing	Field testing	TWP
B.1.b	Stored products pests	N/A	Grain	Develop bioassay	Screening	Isolation	ID	Field evaluation	WB TWP
B.1.c	Pea weevil	N/A	Peas	Isolate & identify by GC/MS		Test for attractancy in lab		Field test	SLC
B.1.d	Papaya fruit fly	Volatile chemicals	Papayas	Study fly behavior	Collect volatiles	Lab and field testing of kairomones as attractants and trap synergists			PJL
B.1.e	Cabbage looper	Volatile chemicals	Cabbage	Isolate and identify kairomones using lab bioassay			Field test synthetic lures		PJL
B.1.f	*H. zea, H. virescens*	Volatile chemicals	Host plants	Lab and field observation on host odor effects		Isolate and identify compounds			PJL
B.1.g	*H.zea*	N/A	Willow and Dallisgrass	Develop windtunnel bioassay	Extraction studies	GC isolation & bioassay	ID attractants	N/A	JDW
B.1.h	Hawaiian fruit flies	N/A	Butterfly bush	Establish windtunnel bioassay	Extraction studies	GC isolation & bioassay	ID attractants	N/A	JDW
B.1.i	Hawaiian fruit flies	Plant volatiles, terpenoids, sesquiterpenoids	Host plants, papaya, essential oils	Isolate compounds for bioassay	Test individual compounds	Seek economical sources	Prepare synthetic mixtures for bioassay	N/A	RAF
B.1.j	Japanese beetle	Unknown	Host & other plants	Field screening of candidate attractants	Continue screening & evaluate selected materials	Develop traps & lure dispensing devices	Large field tests with best traps & lures	Evaluate population suppression with traps & lures	MGK
B.1.k	*H. zea*	Plant volatiles	Adult food plants	Isolate & identify chemicals for use in attracticide formulation	Test for attractancy in lab	Synthesize chemicals & formulate for field tests	Formulate attractants with feeding stimulants and toxicants	Field test	TNS KRB PDL

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.1 ATTRACTANTS (Cont.)									
B.1.l	*H. zea*	Plant volatiles	Adult food plants	Isolate & identify chemicals	Isolate & identify chemicals; test for attractancy	Evaluate for trapping efficiency w/ & w/o pheromones	Cont.	Cont.	TNS KRB PDL
B.1.m	Stored grain beetles	N/A	Host insect	Isolate kairomone; develop bioassay	Identify kairomone	Synthesize kairomone	Bioassay synthetic kairomone	Field test synthetic kairomone	RWH
B.1.n	Mexican fruit fly	N/A	Bacteria	Isolate attractive compounds by GC		Identify components by GC-MS	Lab bioassay compounds individually and together		DR
B.1.o	Mexican fruit fly	N/A	Amino acid metabolites & protein baits	Test new compounds in lab bioasssays	Field test new & existing compounds	Formulate optimal mixture into slow-release dispenser	Field test dispenser	Test w/ other tephritids	DR
B.1.p	*Heliothis, Spodoptera*	N/A	Host insect-produced kairomones	Study host finding behavior; obtain active extracts w/ kairomonal activity	Fractionate extracts; isolate active fractions; identify active compounds	Synthesize & formulate active compounds	Integrate kairomones w/ surrogate host for artificial rearing	Scale up for mass rearing	JEC WSS RJH HRG PDG
B.1.q	*Heliothis virescens* females only	N/A	Host plants	Isolate & identify kairomones using lab bioassay			Field test synthetic lures		ERM FCT
B.1.r	*Cucurlio caryae*	N/A	Conspecifics & pecan nut tissue	Develop bioassay	Bioassay & isolate	Bioassay, isolate & characterize			MTS
B.1.s	*Cydia caryana*	N/A	Conspecifics & pecan nut tissue	Develop bioassay	Bioassay & isolate	Bioassay, isolate & characterize			MTS
B.1.t	*Sitotroga cerealella*	N/A	Corn, fungi complexes	Bioassay & isolate			Develop monitoring technology		DKW
B.1.u	*Heliothis virescens*	Volatiles	*Nicotiana spp.*	Identify & bioassay					MGS DMJ RFS

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year: 1	2	3	4	5	INV.[a]
B.1 ATTRACTANTS (Cont.)									
B.1.v	Tephritid fruit flies	Terpenoids	Plants	Screening	Isolation	ID	Field testing	Technology transfer	RT
B.1.w	Pecan aphids	N/A	Hickory & pecan	Obtain plant extracts	LC/GC/MS evaluation	Isolation, bioassay, identification	Field test	Field test	RFS MTS
B.1.x	*Cardiochiles nigriceps*	Terpenoids, carbonyls, alcohols	*Nicotiana spp.* other plants	Isolate & ID by GC/MS	Develop wind tunnel bioassay	Compare volatiles from infested & non-infested plants	Bioassay extracts from three tobaccos & compare w/ field observations		RFS MTS
B.1.y	Tephritid fruit fly	N/A	Protein baits, bacteria, bird feces, host plants	Isolate and identify	Field testing	Field testing	Technology transfer		RRH
B.2 REPELLENTS									
B.2.a	*Sitotroga cerealella*	N/A	Corn, fungi complexes	Bioassay & isolate			Develop monitoring technology		DKW
B.2.b	Stored product insects	Various	Plants & Insects	Screen	Formulate in food packages	Lab evaluate	Field test	Operational tests	TWP WEB
B.3 ANTIFEEDANTS									
B.3.a	Corn rootworm	Antifeedant substances	Corn roots	Estab. & refine feeding bioassay	Fractionate volatile & water sol. root produced subs.	Measure antifeedant activity of fractions; I.D. active subs.	Survey exotic corn germplasm for biol. act. subs.	Determine metabol. pathway of active subs.	WER
B.3.b	Sweetpotato white fly	Azadirachtin	Neem	Lab screening	Plot trials	Assess impact on beneficials	Integrate w/ other agents	Evaluate technology	DA

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.3 ANTIFEEDANTS (Cont.)									
B.3.c	Japanese beetle	Azadirachtin	Neem	Lab screening of candidate repellents	Field tests with most promising materials	Evaluate techniques to dispense repellents	Large scale field tests w/ best systems	Incorporate repellents into IPM systems	MGK
B.3.d	Pecan aphids (3 spp)	N/A	Pecan cultivars, hickory, walnut	Develop bioassay	Bioassay & isolate	Bioassay, isolate & characterize			MTS
B.3.e	Corn rootworm	Antifeedant substances	Corn roots	Establish & refine bioassay	Fractionate volatile & water sol. root-prod. substances	Bioassay fracts., ID antifeed. subs.	Survey exotic germplasm for biol. act. subs.	Determine metabol. pathway of active subs.	WER
B.3.f	*H. zea, Carpophilus hemipterus*	Indole alkaloids	Fungal sclerotia	Bioassay, isolate & characterize					DTW JBG PFD
B.3.g	*H. zea, Spodoptera frugiperda*	Flavone	Corn silks	Isolate & identify compounds	Test compounds in lab bioassays	Determine genetic control, breeding for increased nat. prod.	Stabilize nat. prod. containing germplasm as inbreds	Field test, release of inbreds to seed companies	MES NWW BRW
B.4 PHAGOSTIMULANTS & OVIPOSITION STIMULANTS									
B.4.a	Medfly	Sesquiterpene	N/A	Establish oviposition bioassay	Degradation studies	Isolate & bioassay degradation prods.	ID active components	N/A	JDW
B.4.b	*O. nubialis*	Terpenoids	Host plants	Establish oviposition bioassay	Discover activity	Isolate & identify bioactive compounds - structure, activity relationships			BFB
B.4.c	*Heliothis virescens*	Volatiles	Nicotiana spp.	Identify & bioassay					MGS DMJ RFS
B.4.d	*Helicoverpa zea*	N/A	Corn silk	Bioassay & fractionate	Continue	Identify germplasm w/o stimulant	Field test		RFS
B.4.e	Sweetpotato white fly	N/A	Tobacco, soybean, sweetpotato	Screen plant material	Fractionate	Bioassay	Field test & develop resistant germplasm		RFS

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.4 PHAGOSTIMULANTS & OVIPOSITION STIMULANTS (Cont.)									
B.4.f	*H. zea*	Nectar, honeydew	Adult food plants and sucking insects	Bioassay plant and insect products for feeding response	Bioassay & Identify feeding stimulants	Formulate feeding stimulants with biologically active materials for use in field		Conduct field evaluations w/ attracticides	JDL TNS PDL
B.5 TOXICANTS									
B.5.a	*Acarapis woodi, Varroa jacobsoni*	N/A	N/A	Screen smoke of various plants	Continue screening	Extract active plants	Begin chemical ID	Continue ID	FAE
B.5.b	*Bemisia tabaci*	Sucrose ester	Tobacco	N/A	N/A	N/A	N/A	N/A	GWP RFS
B.5.c	Stored products pests	N/A	Grain	Develop bioassay	Screening	Isolation	ID	Field evaluation	WB
B.5.d	Pear psylla	Sugar ester	*Nicotiana*	Field evaluation	Effect on non-target organisms	Evaluate new plant derivatives	Evaluate new plant derivatives	Field testing new compounds	GJP RFS
B.5.e	Grain Pests	Proteins	Grains & other sources	Isolate and bioassay protein	Isolate and bioassay protein	Isolate gene	Transform plant & microbe	Bioassay	KJK
B.5.f	N/A	Acetogenins	Plants	Identify bioactive constituents & improve extraction		Isolate materials	Study toxicology	N/A	JLM
B.5.g	Western corn rootworm	N/A	Leafy spurge	Establish WCR and leafy spurge colonies	Extraction studies	Isolate toxicants and bioassay	ID toxicants	N/A	JDW
B.5.h	N/A	N/A	N/A	New project					SBS JAD
B.5.i	Sweetpotato whitefly	Acyl sugars	*Nicotiana*	N/A					JWN RFS
B.5.j	Insects generally	N/A	Vegetables	Extraction, isolation, identification, bioassays, continuous process					JKP HFH

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV.[a]
B.5 TOXICANTS (Cont.)									
B.5.k	Sweetpotato, aphids	Sugar esters, alkaloids	*Nicotiana spp.*	Synthesize	Bioassay, evaluate effects on non-target organisms	Develop CRADA & field tests	Develop commercial products		RFS OTC DMJ
B.5.l	Sweetpotato	Alkaloids	Sweetpotato, tobacco, squash, etc.	N/A					RJH
B.5.m	Caribbean fruit flies	Flavonoids	Citrus peel	Bioassay	Bioassay and isolate	Bioassay, isolate and characterize			JPS RTM
B.5.n	Lepidopterans	N/A	Plants	Bioassay and isolate bioactive constituents; investigate mode of action; scaleup, economic assessment of production potential					DMG
B.5.o	Lepidopterans, tephritid & muscoid flies	N/A	Entomogenous fungi	Bioassay and isolate bioactive constituents; investigate mode of action; scaleup, economic assessment of production potential					SBK DMG
B.5.p	Root lesion nematode; golden cyst nematode	N/A	Plants	Bioassay & isolate bioactive constituents; investigate mode of action; scaleup, economic assessment of production potential					DMG
B.5.q	Root lesion nematode; golden cyst nematode	N/A	Fungi	Bioassay & isolate bioactive constituents; investigate mode of action; scaleup, economic assessment of production potential					DMG
B.5.r	Mites in pet food	Terpenoids, others	Plants & insects	Screening	Formulate	Lab evaluate	Field test	Field test	TWP
B.5.s	*Heliocoverpa zea*, *Heliothis virescens*	*Nicotiana spp.* other plants	Screen plant material	Fractionate , bioassay, identify		Field test & develop resistant germplasm			RFS
B.5.t	Grain insects	Proteins	Grain	Evaluate bioassays			Continue		JEB
B.5.u	Parasitoids (beneficial insects)	N/A	Grain, other	Bioassays, isolation			Identify, application		JEB
B.5.v	Citrus root weevil	Pyranocoumarins	Citrus roots	Characterize	Bioassay, isolate and characterize				JPS

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.6 GROWTH REGULATORS									
B.6.a	Lepidoptera, coleoptera	Venoms, toxins	Parasitic wasps	Isolate toxin	Isolate toxin	Engineer toxins	Engineer toxins	Define mode of action	TAC
B.6.b	Sweetpotato white fly	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCC
B.6.c	Caribbean fruit fly	Gibberellic acid	Gibberella fujikuroi	Determine effects of GA on citrus peel components					PES
B.6.d	Lepidopterans	Insect growth regulators	N/A	Refinement of formulations and ID of new compounds; evaluate in field under EUP; integrate w/ other controls; expand into area-wide tests.					LDC
B.6.e	Noctuids	Neem, limonoids, capsaicin	N/A	ID activity & bioassay in lab; field evaluate activity; scale up; integrate with other control strategies					LDC
B.6.f	Tephritid fruit flies	Gibberellic acid	Gibberella fujikuroi	Pilot test gibberellic acid application methods and evaluate effects on fruit senescence and fruit fly susceptibility					PDG REM WJS PES
B.6.g	*H. zea*	Precocene, juvenile hormones, mimics	Host plants	N/A					BFB
B.f.h	Stored grain pests	Eicosanoids	Insect defense secretions	Select physiol. systems., design bioassays.	Identify eicosanoids & new inhibitors	Bioassay inhibitors	Develop control strategies	Test control strategies	RWH
B.7 METABOLIC INHIBITORS									
(None Submitted)									
B.8 UNKNOWN - OTHER									
B.8.a	*Heliothis, Helicoverpa*	Steroids, terpenoids	Plants	Screening	Isolation	ID & attempt hybridization			CAE
B.8.b	Lepidopterans	N/A	Insects	N/A					WCN
B.8.c	*Heliothis, Heliocoverpa*	Neuropeptide mimetics	Insects, synthesis	Design pseudopeptide mimetics of insect neuropeptides	Design & computer screen for nonpeptide mimetics of insect neuropeptides			Test mimetics	RJN

RESEARCH AREA B: NATURAL PRODUCTS FOR ARTHROPOD CONTROL

Activity Code	Target Pest	Natural Prod.	Source	Activity During Year 1	2	3	4	5	INV. [a]
B.8 UNKNOWN - OTHER (Cont.)									
B.8.d	Mosquitoes, livestock insect	Neuropeptide mimetics	Insects, synthesis	Design pseudopeptide mimetics of insect neuropeptides	Design pseudopeptide mimetics of insect neuropeptides / Design & computer screen for nonpeptide mimetics of insect neuropeptides	Design & computer screen for nonpeptide mimetics of insect neuropeptides	Design & computer screen for nonpeptide mimetics of insect neuropeptides	Test mimetics	RJN
B.8.e	Sweetpotato white fly	Protein	Cucurbits	Identify proteins	Isolate and characterize	Isolate and characterize	Isolate genes	Isolate and clone genes	JPS

a) Investigator Code: See list with name and location at the end of this Research Area.

b) Not applicable, not provided, or not relevant.

INVESTIGATOR CODES
RESEARCH AREA B

CODE	NAME, LOCATION
DA	D. Akey, Phoenix, AZ
KRB	K.R. Beerwinkle, College Station, TX
BFB	B.F. Binder, Ames, IA
WB	W. Burkholder, Madison, WI
JEC	J.E. Carpenter, Tifton, GA
LDC	L.D. Chandler, Tifton, GA
OTC	O.T. Chortyk, Athens, GA
CCC	C.C. Chu, Brawley, CA
SLC	S.L. Clement, Pullman, WA
TAC	T.A. Coudron, Columbia, MO
PFD	P.F. Dowd, Iowa City, IA
JAD	J.A. Duke, Beltsville, MD
FAE	F.A. Eischen, Weslaco, TX
CAE	C.A. Elliger, Albany, CA
RAF	R.A. Flath, Albany, CA
JBG	J.B. Gloer, Iowa City, IA
PDG	P.D. Greany, Gainesville, FL
HRG	H.R. Gross, Tifton, GA
HFH	H.F. Harrison, Charleston, SC
RRH	R.R. Heath, Gainesville, FL
RJH	R.J. Horvat, Athens, GA
RWH	R.W. Howard, Manhattan, KS
DMJ	D.M. Jackson, Oxford, NC
MGK	M.G. Klein, Wooster, OH
KJK	K.J. Kramer, Manhattan, KS
PJL	P.J. Landolt, Gainesville, FL
PDL	P.D. Lingren, College Station, TX
JDL	J.D. Lopez, Jr., College Station, TX
RTM	R.T. Mayer, Orlando, FL
REM	R.E. McDonald, Orlando, FL
JLM	J.L. McLaughlin, West Lafayette, IN
JWN	J.W. Neal, Beltsville, MD
WCN	W.C. Nettles, Jr., Weslaco, TX
JKP	J.K. Peterson, Charleston, SC
TWP	T.W. Phillips, Madison, WI
GWP	G.W. Pittarelli, Beltsville, MD
WER	W.E. Riedell, Brookings, SD
DR	D. Robacker, Weslaco, TX
WSS	W.S. Schlotzhauer, Athens, GA
WJS	W.J. Schroeder, Orlando, FL
RFS	R.F. Severson, Athens, GA
JPS	J.P. Shapiro, Orlando, Fl
TNS	T.N. Shaver, College Station, TX
PES	P.E. Shaw, Winter Haven, FL
MTS	M.T. Smith, Stoneville, MS
SBS	S.B. Sternberr, Beltsville, MD
RT	R. Teranishi, Albany, CA

INVESTIGATOR CODES (CONT.)
RESEARCH AREA B

CODE	NAME, LOCATION
DTW	D.T. Wicklow, Iowa City, IA
JDW	J.D. Warthen, Jr., Beltsville, MD

RESEARCH AREA C

Natural Products for Parasite and Pathogen Control

RESEARCH AREA C: NATURAL PRODUCTS FOR PARASITE AND PATHOGEN CONTROL

Activity Code	Target Pest	Natural Prod.	Source	ACTIVITY DURING YEAR 1	2	3	4	5	Investigators[a]
C.1 Fungicides									
C.1. a	*Aspergillus flavus*	Phenolic, oils, soaps, waxes	Plant, fungal	Bioassay, isolate, identify	Bioassay, isolate, identify	Bioassay, isolate, identify	Evaluate commer-icialize	Evaluate commer-icialize	DB, RAN, HJZ
C.1. b	*A. parasiticus*	Phenolic, oils, soaps, waxes	Plant, fungal	Bioassay, isolate, identify	Bioassay, isolate, identify	Bioassay, isolate, identify	Evaluate commer-icialize	Evaluate commer-icialize	DB, RAN, HJZ
C.1. c	*Armillaria spp.*	Multiple	Fungal, bacterial	Isolate, identify	Isolate, identify	Evaluate bioassays	Evaluate bioassays	Evaluate bioassays	HGC
C.1. d	*Botrytis cinerea*	Pyrrolnitrin, phenolic, oils,soaps,waxes	Plant, bacterial	Bioassay	Bioassay	Patent license	Patent license	Patent license	SFV, CLW
C.1. e	*Cercospora beticola*	Flavonoid	Plant	Greenhouse studies	Greenhouse studies	Greenhouse and field studies	Field studies	Field studies	SSM
C.1. f	*Cladosporium caryigenum*	Phenolic	Plant	Identify, isolate	Identify, isolate	Bioassays	Bioassays and field studies	Field studies	IEY
C.1. g	*Colletotrichum spp.*	Phenolic, flavonoid	Plant	Bioassays	Bioassays	Evaluate, develop for commercial	Evaluate, develop for commercial	Evaluate, develop for commercial	DNO, SFV
C.1. h	*Erysiphe spp.*	Oils,soaps,waxes	Plant	Evaluate	Evaluate	Develop for commercialization	Develop for commercialization	Develop for commercialization	MC
C.1. i	*Fusarium spp.*	Terpenoid, pyrrolnitrin, phenolic, glucosinolate	Plant, fungal, bacterial	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Evaluate, commercialize	Evaluate, commercialize	Many
C.1. j	*Monilinia fructicola*	Acids,esters	Plant, bacterial	Mode of action studies	Mode of action studies	Application	Application, field trials	Field trials	JLS, DAM
C.1. k	*Puccinia spp.*	Oils,soaps,waxes	Plant	Evaluate	Evaluate	Develop for commercialization	Develop for commercialization	Develop for commercialization	MC
C.1. l	*Pythium spp.*	Terpenoid, steroids, other	Plant, fungal	Identify, evaluate, determine mechanisms of action	Identify, evaluate, determine mechanisms of action	Identify, evaluate, determine mechanisms of action	Identify, evaluate, determine mechanisms of action	Identify, evaluate, determine mechanisms of action	Many
C.1. m	*Rhizoctonia spp.*	Glucosinolate, terpenoid, steroids	Plant	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Evaluate, commercialize	Evaluate, commercialize	RDL, DF, SFV
C.1. n	*Verticillium spp.*	Terpenoid, phenolic, glucosinolate	Plant, fungal	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Evaluate, commercialize	Evaluate, commercialize	Many

RESEARCH AREA C: NATURAL PRODUCTS FOR PARASITE AND PATHOGEN CONTROL

Activity Code	Target Pest	Natural Prod.	Source	ACTIVITY DURING YEAR 1	2	3	4	5	Investigators[a]
C.1 Fungicides (Cont.)									
C.1. o	Fungi - Undefined spp.	Peptide, alkaloid, flavonoid, phenolic	Plant, fungal, bacterial	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Evaluate, commercialize	Evaluate, commercialize	Many
C.2 Nematocides									
C.2. a	*Heterodera spp.*	Glucosinolate	Plant	Greenhouse studies bioassays	Greenhouse studies bioassays	Greenhouse studies field studies	Field studies	Field studies	SLFM, SSM
C.2. b	*Meloidogyne spp.*	Glucosinolate, phenolic	Plant, nematode	Greenhouse studies bioassays	Greenhouse studies bioassays	Greenhouse studies formulation studies	Formulation studies	Formulation studies	FSS, AFR
C.2. c	*Rotylenchus reniformis*	Glucosinolate	Nematode	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay	AFR
C.2. d	Nematodes - General	Phenolic	Plant	Identify, bioassay	Identify, bioassay	Identify, bioassay	Identify, bioassay, commercialize	Identify, bioassay, commercialize	GJB, JC, RFS, RPS
C.3 Bacteriocides									
C.3. a	*Erwinia spp.*	Other	Plant, fungal, bacterial	Evaluate	Evaluate, process, develop	Commercialize	Commercialize	Commercialize, test market	JL
C.3. b	Bacteria - General	Peptide	Plant	Identify, isolate, bioassay	Identify, isolate, bioassay	Identify, isolate, bioassay, develop commercial	Identify, isolate, bioassay, develop commercial	Identify, isolate, bioassay, develop commercial	GJB, FAH, RJH LDO, RPS
C.4. Physical Barriers									
C.4. a	Fungi *Fusarium sambucinum*	Phenolic, oils,soaps,waxes	Plant	Study mechanism of action of defense barriers; evaluate cultivars and germplasm					ECL, PHO, SFV
C.4. b	Bacteria *Erwina carotovora*	Phenolic, oils,soaps,waxes	Plant	Understand plant resistance mechanisms; modify cultural practices					ECL, PHO

a. Investigators Code: See list with Investigator name and location at the end of this Research Area.

INVESTIGATOR CODES
RESEARCH AREA C

CODE	NAME, LOCATION
*AAB	A.A. Bell, College Station, TX
DB	D. Bhatnagar, New Orleans, LA
GJB	G.J. Bethlenfalvay, Corvallis, OR
*JMB	J.M. Bland, New Orleans, LA
*KDB	K.D. Burkhead, Peoria, IL
MC	M. Carter, Beltsville, MD
*AMC	A.M. Collins, Weslaco, TX
*WJC	W.J. Connick, Jr., New Orleans, LA
HGC	H.G. Cutler, Athens, GA
*DDD	D.D. Daigle, New Orleans, LA
*JAD	J.A. Duke, Beltsville, MD
*CPD	C.P. Dionigi, New Orleans, LA
*PE	P. Engel, New Orleans, LA
DF	D. Fravel, Beltsville, MD
FAH	F.A. Hammerschlag, Beltsville, MD
*HFH	H.F. Harrison, Charleston, SC
RJH	R.J. Horvat, Athens, GA
*CRH	C.R. Howell, College Station, TX
*SEK	S.E. Kunz, Kerrville, TX
*JCL	J.C. Locke, Beltsville, MD
*JL	J. Loper, Corvallis, OR
ECL	E.C. Lulai, East Grand Folks, MN
RDL	R.D. Lumsden, Beltsville, MD
DAM	D.A. Margosan, Fresno, CA
SSM	S.S. Martin, Fort Collins, CO
*SM	S. McCormick, Peoria, IL
SLFM	S.L.F. Meyer, Beltsville, MD
*RAM	R.A. Moreau, Philadelphia, PE
RAN	R.A. Norton, Peoria, IL
DNO	D.N. O'Neill, Beltsville, MD
LDO	L.D. Owens, Beltsville, MD
*JP	J. Paxton, Univ. of IL, IL
*JKP	J.K. Peterson, Charleston, SC
*RGP	R.G. Powell, Peoria, IL
*PLP	P.L. Pusey, Byron, GA
*AFR	A.F. Robinson, College Station, TX
*FSS	F.S. Santamoru, Jr. Washington, DC
RPS	R.P. Schreiner, Corvallis, OR
RFS	R.F. Severson, Athens, GA
*PJS	P.J. Slininger, Peoria, IL
*JLS	J.L. Smilanick, Fresno, CA
*SBS	S.B. Sternberr, Beltsville, MD
*RDS	R.D. Stipanovic, College Station, TX
SFV	S.F. Vaughn, Peoria, IL
CLW	C.L. Wilson, Kearneysville, WV
*WTW	W.T. Wilson, Weslaco, TX

INVESTIGATOR CODES (CONT)
RESEARCH AREA C

CODE	NAME, LOCATION
*IEY	I.E. Yates, Athens, GA
HJZ	H.J. Zeringue, Jr. New Orleans, LA

* Included under Investigators as "Many."

RESEARCH AREA D

Natural Products for Weed Control

RESEARCH AREA D. NATURAL PRODUCTS FOR WEED CONTROL

				Activity During Year					
Activity Code	Activity	Natural Prod.	Source	1	2	3	4	5	Investigators [a]
D1. Microorganisms									
D.1. a	Accession of microorganisms and primary bioassays	Microbial phytotoxins	Micro-organisms, bacteria, fungi	Access, lyo, identify microorganisms, ferment, assay	Ferment, identify compounds, assay	Ferment, identify compounds, assay	Bioassay in advanced systems	Develop agreements with industry and field test	HGC, HKA, RJK
D. 1. b	Fermentation of promising microorganisms	Microbial phytotoxins	Micro-organisms, bacteria, fungi	Determine best ferment system	Ferment, isolate important compounds	Ferment isolate bulk quantities	Ferment isolate bulk quantities	Large fermentation, indust. cooperation	HGC, HKA, RJK
D2. Plants									
D.2 a	Accession of plants and growing for phytotoxin production	Plant secondary metabolites, phytotoxins	Plants, monocots, dicots	Access plants extract	Identify compounds assay	Assay in multiple systems	Grow plants for bulk metabolite isolation	Develop with industry	JB, DRG, JL, JKP, HFH, SBS, JAD, RB, RJS
D3. Microorganisms/Plants									
D.3. a	Isolation and identification of biologically active substances	Biologically active natural products	Micro-organisms, plants	Isolation of natural products from diverse sources	UV, IR, NMR, Mass Spec, Fab MS, X-ray	UV, IR, NMR, Mass Spec, Fab MS, X-ray	Obtain specific activity, target spec. relative to structure	Agreements with industry	HGC, HKA, RJS
D4. Bioassays									
D.4. a	Bioassays and secondary screens of purified compounds	Biologically active natural products	Micro-organisms, plants	Complete primary bioassay	Start secondary greenhouse tests	Greenhouse and field tests	Field tests on target organisms	Co-develop with industry	SOD, DRG, JL, JB, PHO, REH
D5. Structure Activity Relations (Sar)									
D.5. a	Mode of action and SAR	Biologically active natural products	Micro-organisms, plants	Preliminary SAR in plants and microorganism	Secondary SAR in plants and microorganisms	Tertiary SAR in plants and organism	Derivative testing in plants and organisms	Terminal publication	SOD, DRG, JL, JMB, (SAR only) - JB, REH
D6. Field Tests									
D.6. a	Field testing	Biologically active natural products	Micro-organisms, plants	General field tests for phytotoxicity and other props	Specific crop testing	Small block tests	Regional block tests	National and International field testing	DRG, SOD, JL

a. Investigators Codes: See list with investigator name and location at the end of this Research Area.

INVESTIGATOR CODES
RESEARCH AREA D

CODE	NAME, LOCATION
HKA	H.K. Abbas, Stoneville, MS
JMB	J.M. Bland, New Orleans, LA
RB	R. Boydston, Prosser, WA
JB	J. Bradow, New Orleans, LA
HGC	H.G. Cutler, Athens, GA
JAD	J.A. Duke, Beltsville, MD
SOD	S.O. Duke, Stoneville, MS
DRG	D.R. Gealy, Stuttgart, AR
HFH	H.F. Harrison, Charleston, SC
REH	R.E. Hoagland, Stoneville, MS
RJK	R.J. Kremer, Columbia, MO
JL	J. Lydon, Beltsville, MD
PHO	P.H. Orr, E. Grand Forks, MN
JKP	J.K. Peterson, Charleston, SC
RJS	R.J. Smeda, Stoneville, MS
SBS	S.B. Sternberr, Beltsville, MD

RESEARCH AREA E

Delivery Systems/Application Tech. for Natural Products

RESEARCH AREA E: DELIVERY SYSTEMS/APPLICATION TECHNOLOGY

Activity Codes & Research Approaches	Activity During Year 1	2	3	4	5	
	Field Experiments	Field Experiments	Field Experiments	Field Experiments	Field Experiments	Investigators [a]
E.1. Measure drift from orchard	Develop or obtain 2 dispersion models	Modify models for orchard conditions	Compare models with experiments	Improve models, make specific experiments to test problem variables	Final testing of orchard dispersion models	All
E.2. Measure physical properties of spray solutions	Develop more accuracy in test methods, compare measured values with spray results	Evaluate a range of additives for spray results and dynamic properties	Compile data base of spray mix, physical and droplet characteristics			JEM, MAL,DR
E.3. Use EBA to analyze spray deposit	Laboratory evaluation of tracer, target interactions	Establish procedures for field eval. of spray droplet deposition	Test spray mix, deposit location, deposit type	Complete experiment: report results on droplet deposits		WJC, DDD, DR, ODD, RMJ, ABP, BSS, MRM
E.4. In line mixing	Lab experiments on mixing time scale	Establish procedures and test formulations including adjuvant effects	Complete experiment: Results on required mixing and injection points for various formulations			JAM, RDF, HRS, IWK, KDH
E.5. Plant stress	Greenhouse experiments of effect of stress on spray droplet, leaf interaction	Evaluate effect of stress level on uptake of selected material	Experiments to determine critical stress level for selected materials	Complete experiments on plant stress effect on uptake of foliar applied materials		SEK, JAM, DHA, RDF, HRS, IWK, MAL, JEM, KDH, DR
E.6. Delivery techniques					Determine effect of delivery techniques on environmental fate of nat. prod.	ODD, RMJ, ABP

1. Investigator Codes: See list with investigator name and location at the end of this Research Area.

INVESTIGATOR CODES
RESEARCH AREA E

CODE	NAME, LOCATION
DHA	D.H. Akey Phoenix, AZ
WJC	W.J. Connick, Jr., New Orleans, LA
DDD	D.D. Daigle, New Orleans, LA
ODD	O.D. Dailey, New Orleans, LA
RDF	R.D. Fox, Wooster, OH
KDH	K.D. Howard, Stoneville, MS
RMJ	R.M. Johnson, New Orleans, LA
IWK	I.W. Kirk, College Station, TX
SEK	S.E. Kunz, Kerrville, TX
MAL	M.A. Latheef, College Station, TX
MRM	M.R. McGuire, Peoria, Il
JAM	J.A. Miller, Kerrville, TX
JEM	J.E. Mulrooney, Stoneville, MS
ABP	A.B. Pepperman, New Orleans, LA
DR	D. Robacker, Weslaco, TX
BSS	B.S. Shasha, Peoria, Il
HRS	H.R. Sumner, Tifton, GA

APPENDICES

APPENDIX A

ARS Working Conference on Natural Products
for Control of Agricultural Pests

WORKSHOP AGENDA

Richard B. Russell Research Center
Athens, GA
October 19-20, 1993

Monday, October 18 Travel Day

1:00 - 6:00 PM	On-Site Registration/Informal Center Tours
5:00 - 6:00 PM	Steering Committee, National Program Staff, Moderators and Session Leaders Meeting--R.M. Faust

Tuesday, October 19 Morning

7:00 - 8:00 AM	On-Site Registration
8:00 - 8:10 AM	Call to Order and Administrative Details -- H.G. Cutler
8:10 - 8:25 AM	Welcome to the Working Conference -- M.E. Carter Welcome to the Russell Center -- F.C. Greene
8:25 - 8:50 AM	Overview and Objectives and Charge to the Working Conference -- R.M. Faust

PLENARY SESSION

Moderator: H. G. Cutler

8:50 - 9:20 AM	Challenges and Needs for Natural Products to Control Agricultural Pests -- R.H. Villet, OTT The ARS Patent Process--Gail E. Poulos, OTT

9:20 - 9:50 AM Opportunites for Extramural Cooperation: The RIPS-ARS Connection -- J.D. McChesney

9:50 - 10:20 AM Natural Products: The Perspective of the Agricultural Chemical Industry -- Carl Snipes, Dow Elanco

10:20 - 10:40 AM Break

10:40 - 11:25 AM Regulatory Considerations

Regulatory Considerations for Natural Products: Guidelines for ARS -- R.M. Parry

Regulatory Considerations for Natural Products: Improving the Process -- A.E. Lindsay

Natural Products: The IR-4 Program -- N. P. Thompson

11:25 - 12:25 PM Natural Product Technology Transfer Opportunities: Industrial Representatives

Leader: E. Sanders, Monsanto

Participants:
A. Duggan, FMC
M. Los, American Cyanamid
C. Snipes, Dow Elanco
V. Illum, CTT Corp.

12:25 - 1:25 PM Lunch Break

RESEARCH AREA PANEL DISCUSSIONS

1:25 - 2:40 PM A. Chemical Methodologies and Bioassays for Natural Products

Leader: Dr. R. Chen, Abbott Co.

Participants:
L. Anderson
G. Elzen
J. King
G. Leather
G. Piterelli

- Summary Status of Research Area in ARS
- Needs and Priorities
- Program Links
- Recommendations

2:40 - 2:50 PM Break

2:50 - 4:05 PM B. Natural Products for Arthropod Control

Leader: P. Greany

Participants: W. Burkholder
R. Heath
J. Neal
J. Shapiro

- Summary Status of Research in ARS
- Needs and Priorities
- Program Links
- Recommendations

4:05 - 4:20 PM Break

4:20 - 5:35 PM C. Natural Products for Parasite and Pathogen Control

Leader: S. Vaughn

Participants: S. Kunz
J. Locke
P. Slininger
C. Wilson

- Summary Status of Research Area in ARS
- Needs and Priorities
- Program Linkages
- Recommendations

5:35 PM Informal Discussions

Wednesday, October 20 Morning

RESEARCH AREA PANEL DISCUSSIONS

8:00 - 9:15 AM — D. Natural Products for Weed Control

Leader: S. Duke

Participants:
H. Abbas
J. Bradow
H. Cutler
D. Gealy
R. Hoagland
J. Lydon

- Summary Status of Research Area in ARS
- Needs and Priorities
- Program Linkages
- Recommendations

9:15 - 9:25 AM — Break

9:25 - 10:40 AM — E. Delivery Systems/Application Technology for Natural Products

Leader: L.F. Bouse

Participants:
R. Fox
K. Howard
A. Pepperman
B. Shasha
H. Sumner
A. Miller

- Summary Status of Research Area in ARS
- Needs and Priorities
- Program Linkages
- Recommendations

10:40 - 10:50 AM — Break

10:50 - 12:00	RESEARCH AND ACTION PLAN CONSENSUS PROCESS/WORK SESSION Instructions for Breakout Groups -- H.G. Cutler

Breakout Groups

Group A: Chemical Methodologies and Bioassays for Natural Products Leader: R. Chen Recorder: R. Chen
Group B: Natural Products for Arthropod Control Leader: P. Greany Recorder: J. Shapiro
Group C: Natural Products for Parasite and Pathogen Control Leader: S. Vaughn Recorder: S. Vaughn
Group D: Natural Products for Weed Control Leader: S. Duke Recorder: R. Hoagland
Group E: Delivery Systems/Applications Technology for Natural Products Leader: L. F. Bouse Recorder: A. Pepperman

12:00 - 1:00 PM	Lunch Break

Wednesday, October 20 Afternoon

1:00 - 2:00 PM	Work Session Continued and Preparation of Summary Reports
	Summary Reports
2:00 - 2:15 PM	A. Chemical Methodologies and Bioassays for Natural Products Reporter: R. Chen
2:15 - 2:30 PM	B. Natural Products for Arthropod Control Reporter: J. Shapiro
2:30 - 2:45 PM	C: Natural Products for Parasite and Pathogen Control Reporter: S. Vaughn

2:45 - 3:00 PM	D: Natural Products for Weed Control Reporter: S. Duke
3:00 - 3:15 PM	E. Delivery Systems/Application Technology for Natural Products Reporter: A. Pepperman
3:15 - 3:30 PM	Break
3:30 - 3:45 PM	General Discussion Moderator: H.G. Cutler
3:45 - 4:00 PM	Concluding Remarks and Adjournment of Conference W. Martinez
4:00 - 5:00 PM	Wrap up for Steering Committee and National Program Leaders R.M. Faust

APPENDIX B

ATTENDEE LIST

**ARS NATURAL PRODUCTS WORKING CONFERENCE
FOR CONTROL OF AGRICULTURAL PESTS
Richard B. Russell Center
Athens, GA
October 19-20 1993**

H. Abbas, USDA, ARS, MSA
Jamie Whitten Delta St. Res. Ctr.
PO Box 350
Stoneville, MS 38776 — 601-686-5313 FAX -5422

Sean Adams
ARS Information Staff
6303 Ivy Lane
Greenbelt, MD — 301-344-2723 FAX-2311

D. Akey
USDA, ARS, PWA
4135 E. Broadway
Phoenix, AZ 85040 — 602-379-3524 FAX -4509

J. Anderson
USDA, ARS, PSI, BARC-W
10300 Baltimore Blvd.
Beltsville, MD 20705-2350 — 301-504-6537 FAX -6491

L. Anderson, USDA, ARS
Univ. of California
Robbins Hall, Rm. 210
Davis, CA 95616 — 916-752-6260 FAX-5410

J. Baker
USDA, ARS, SAA
3401 Edwin St.
Savannah, GA 31405 — 912-651-3544 FAX-3500

B. Binder, USDA
Genetics Lab.C/O Insect. Bldg.,
Iowa State Univ
Ames, IA 50011 — 515-294-6948 FAX -2265

M. Blum
Dept. of Entomology
Univ. of Georgia
Athens, GA 30602 — 706-542-2301 FAX -2279

L. Bouse, USDA, ARS
2331 Scoates Hall, TAMU.
College St., TX 77843 | 409-260-9364 FAX -9367

J. Bradow, USDA, ARS
P.O. Box 19687 Rm. 3030
New Orleans, LA 70179-0687 | 504-286-4479 FAX -4419

F. Bryant, USDA, ARS
P.O. Box 5466
Athens, GA 30613 | 706-546-3378 FAX-3250

K. Burkhead
USDA, ARS, NCAUR
1815 N. University St.
Peoria, IL 61604 | 309-681-6287 FAX -6686

W. Burkholder, USDA, ARS
Dept. of Entomology, MWA
1630 Linden Dr.
Madison, WI 53706 | 608-262-3795 FAX-3322

J. Carpenter, USDA, ARS
P.O. Box 748
Tifton, GA 31793 | 912-387-2343 FAX-2321

M. E. Carter, USDA, ARS
P.O. Box 5677
Athens, GA 30613 | 706-546-3311FAX-3398

M. R. Carter, USDA, USNA
Rm. 208, Bldg. 004, BARC-W
10300 Baltimore Blvd.
Beltsville, MD 20705-2350 | 301-504-6413 FAX-5096

L. Chandler, USDA, ARS
P.O. Box 748
Tiffton, GA 31793 | 912-387-2343 FAX-2321

R. Chen
Dept. 47P, Bldg. AP9A
Abbott Lab.
Abbott Pk. IL 60064 | 708-937-9193 FAX-1021

O. Chortyk, USDA, ARS
P.O. Box 5677-905
College St. Rd.
Athens, GA 30613 | 706-546-3424 FAX-3454

T. Coadron
USDA, ARS
P.O. Box 7629
Columbia, MO 65205-50013   314-875-5361 FAX 4261

H. Cutler
USDA, ARS, RRC, MPRU
P.O. Box 5466
Athens, GA 30613   706-546-3378 FAX-3250

S. Cutler
Mercer University
3001 Mercer University Dr.
Atlanta, GA 30341   404-986-3240 FAX-3384

A. Duggan
External Resources & Screening
P.O. Box 8., Agri. Chem. Div.
Princton, NJ 08540   609-951-3619 FAX-3337

S. Duke, USDA, ARS
Jamie Whitten Delta
P.O. Box 350
Stoneville, MS 38776   601-686-5272 FAX-5422

A. El-Ghaduth
USDA, ARS
45 Wiltshire Road
Kearneysville, WV 25530   304-725-3451 FAX-2340

C. Elliger
USDA, West Reg Res Ctr
800 Buchannan St.
Albany, CA 94710   510-559-5821 FAX-5777

G. Elzen, USDA, ARS
Experiment Stat. Rd.
PO Box 346
Stoneville, MS 38776   601-686-5275 FAX-5421

P. Eugel
USDA, ARS, SRRC
P.O. Box 19687
New Orleans, LA 70179   504-286-4375 FAX-4367

R. Faust
USDA, ARS, NPS
Bldg. 005 BARC-WEST
Beltsville, MD 20783   301-504-6918 FAX-6231

R. Fox
USDA, ARS,
Ohio Agri. Res. & Dev. Ctr.
Wooster, OH 44691 316-263-3871 FAX-3670

N. Fischer
232 Chopin Hall
LA. State Univ.
Baton Rouge, LA 70803-1804 504-388-2695 FAX-3458

D. Gealy, USDA, ARS, PWA
165 Johnson Hall
Washington State University
Pullman, WA 99164-6416 501-673-2661 FAX-4315

P. Greany, USDA, ARS, SAA
1700 SW 23rd Dr.
PO Box 14565
Gainesville, FL 32608 904-374-5763 FAX-5781

F. Greene, USDA, ARS
P. O. Box 5466
Athens, GA 30613 706-546-3541 FAX-3367

L. Harris
USDA, ARS, BARC-W
Bldg 005 Rm 238
Beltsville, MD 20705 301-504-6061 FAX-5467

H. Harrison
USDA, ARS, SAA
2875 Savannah Hwy.
Charleston, SC 29414 803-556-0840 FAX-7013

R. Heath, USDA, ARS, SAA
Research Lab.
1700 SW 23rd Dr.
Gainesville, FL 32608 904-374-5735 FAX-5859

R. Hoagland
USDA, ARS, SWSL
P.O. Box 350
Stoneville, MS 38776 601-686-5210 FAX-5422

K. Howard, USDA, ARS
Jamie Whitten Delta
PO Box 350
Stoneville, MS 38776 601-686-5240 FAX-5422

R. Howard
USDA, ARS
1515 College Ave.
Manhattan, KS 66502 913-776-2706 FAX-2792

V. Illum
CCT Corporation
6613 Haskins
Shawnee, KS 66216 913-268-7504 FAX-same

J. R. King
USDA, ARS, SAA
13601 Old Cutler Rd.
Miami, FL 33158 305-254-3630 FAX-9330

J. W. King
NCAUR- ARS-USDA
1815 N. University
Peoria, IL 61604 309-681-6203 FAX-6686

I. Kirk
231 Scoates TAMU
College Station, TX 77843 409-260-9364 FAX-9367

M. Klein
USDA, ARS, OARDC
Woaster, OH 44691 216-263-3896 FAX-3696

R. Kremer
144 Mumford Hall
University of Missouri
Columbia, MO 65211 314-882-6408 FAX-4960

S. Kunz, USDA, ARS, SPA
2700 Fredrickburg Rd.
Sidney Baker St. AT 1H-10
Kerrville, TX 78028 210-792-0303 FAX-0314

P. Landolt
USDA, ARS
P.O. Box 14565
Gainesville, FL 32604 904-3374-5756 FAX-5781

G. Leather
USDA, ARS, NAA
Fort Detrick, Bldg. 1301, Rm. 136
Frederick, MD 21702 301-619-7330 FAX-2880

A. Lindsay
Dir, Pesticides Prog.
401 M. Street S. W.
Washington, DC 20460 703-305-7102 FAX-6642

J. Locke, USDA, USNA
BARC-W, Bldg. 004, Rm. 208
10300 Baltimore Blvd.
Beltsville, MD 20705-2350 301-504-6413 FAX-5096

M. Los American Cyanamid P.O. Box 400, Agri. Div. Princeton, NJ 08540	609-799-0400,Ext.2547 FAX-1842
E. Lulai USDA, ARS, Pot. Res. Lab. 311 5th Ave. NE P.O. Box 113 East Grand Forks, MN 56721	218-773-2473 FAX-2207
J. Lydon, USDA, ARS Bldg 001, Rm 236 BARC-West 10300 Baltimore Blvd. Beltsville, MD 20705-2350	301-504-53379 FAX-6491
B. Maw Agri. Engineering Research GA. Costal Plain Experiment Station Tifton, GA 31793	912-382-6832
D. Margosan, USDA, ARS Hort. Crops Res Lab 2021 S. Peach Ave. Fresno, CA 93727	209-453-3167 FAX-3088
W. Martinez USDA, ARS, NPS Bldg 005, Rm. 107 BARC-WEST Beltsville, MD 20705	301-504-6275 FAX-6699
J. McChesney Director of RIPS Univ. of Miss. Rm. 411 Fraser University, MS 38677	601-232-7132 FAX-5118
A. Miller USDA, ARS 2700 Fredrickburg Road Kerrville, TX 78028-0914	210-792-0318 FAX-0314
R. Moreau Eastern Reg. Res. Cnt. 600 East Mermaid Lane Philadelphia, PA 19118	215-233-6428 FAX-6559
J. Mulrooney, USDA, ARS Jamie Whitten Delta PO Box 350 Stoneville, MS 38776	601-686-5342 FAX-5422
R. Nachman, USDA, ARS Route 5, Box 810 College Station, TX 77845	409-260-9315 FAX-9377

J. Neal, Jr.
US National Arboretum
10300 Baltimore Blvd. Bldg. 470
Beltsville, MD 20705-2350 301-504-9159 FAX-9097

W. Nettles, Jr.
Subtropical Agr. Res. Lab.
2413 East Hwy 83
Weslaco, TX 78596 210-969-4868 FAX-4888

R. Parry
USDA, ARS
Bldg 005, BARC-WEST
Beltsville, MD 20705 301-504-5734 FAX-5060

A. Pepperman
USDA, ARS, MSA
P.O. Box 19687 Rm 3002
New Orleans, LA 70179 504-286-4510 FAX-4367

J. Peterson, USDA, ARS
Veg Lab
2875 Savannah Hwy
Charleston SC 29414 803-556-0840 FAX-7013

T. Phillips
237 Russell Lab.
Univ. of Wisconsin
Madison, WI 53706 608-262-0814 FAX-3322

G. Pittarelli
USDA, ARS, PSI
BARC-W, Bldg. 009 Rm. 4
Beltsville, MD 20705-2350 301-504-5598 FAX-5867

G. E. Poulos
USDA, ARS, OTT
Bldg. 005, BARC-West
Beltsville, MD 20705 301-504-5345 FAX-5060

R. Powell
Natl Ctr for Agri Utili Res
1815 N. University
Peoria, IL 61604 309-681-6595 FAX-6686

R. Pressey
USDA, ARS, SAA, RRC
950 College St. Rd.
Athens, GA 30613 706-546-3544 FAX-3250

E. Sanders
Monsanto GG3A
700 Chesterfield Pkway North
Chesterfield, MO 63198-0001 314-537-6324 FAX-7125

D. Seigler
265 Morrill Hall
505 S. Goodwin St.
Univ. of Illinois
Urbana, IL 61801 — 217-333-7577 FAX-244-7246

R. Severson
USDA, ARS, RRC
950 College Station Rd.
Athens, GA 30613 — 706-546-3495 FAX-3454

J. Shapiro
USDA, ARS
2120 Camden Rd.
Orlando, FL 32803 — 407-897-7376 FAX-7309

B. Shasha
USDA, ARS, NCAUR
1815 N. University St.
Peoria, IL 61604 — 309-681-6310 FAX-6686

P. Shaw
USDA, ARS, SAA
600 Avenue S. NW
Winter Haven, FL 33881 — 813-293-4133 FAX-8678

L. Shearer
Athens News
One Press Place
Athens, GA 30603 — 706-208-2236 FAX-2246

P. Slininger
USDA, ARS, NCAUR
1815 N. University St.
Peoria, IL 61604 — 309-681-6286 FAX-6686

Michael T. Smith
USDA, ARS, SWSL
P.O. Box 350
Stoneville, MS 38776 — 601-686-5289 FAX-5421

C. Snipes
Dow Elanco, Dis. Research
P.O. Box 708, 2001 W. Main St.
Greenfield, IN 46140 — 317-337-3172 FAX-3205

Omelio Sosa, Jr.
Star Route Box 8
Camal Point, FL 33438 — 407-924-5227 FAX-6109

M. Stephenson
Nematodes, Weeds & Crop Res.
P.O. Box 748
Tifton, GA 31793 — 912-386-3167 FAX-7225

R. Stipanovic
Cotton Pathology Res. Unit
Rt. 5, Box 805
College St., TX 77845 409-260-9232 FAX-9333

H. Sumner, USDA, ARS
GA. Costal Plains, Exp. Sta.
PO Box 748
Tiffton, GA 31793 912-387-2347 FAX-2321

S. Taylor
NCAUR, ARS, USDA
1815 N. Univ. St.
Peoria, IL 61604 309-681-6204 FAX-6686

N. Thompson
Pest. Res. Lab., Univ of Fla
23rd Dr. P.O. Box 110720
Gainsville, FL 32611 904-392-1978 FAX-1988

S. Vaughn, UDSA, ARS
MWA, N. Reg. Res. Ctr.
1815 N. University
Peoria, Il 61604 309-681-6344 FAX-6686

R. H. Villet
USDA, ARS, NPS
Bldg 005, BARC-WEST
Beltsville, MD 20705 301-504-5345 FAX-5060

D. Warthen
Bldg. 007, Rm 312 BARC-W
10300 Baltimore Blvd.
Beltsville, MD 20705-2350 301-504-6981 FAX-6580

D. Weaver, USDA, ARS
3401 Edwin St.
Savannah, GA 31405 912-652-4398 FAX-3500

C. Wilson, USDA, ARS
Appalachain Fruit Res. St.
45 Wiltshire Rd.
Kearneysville, WV 25430 304-725-3451 FAX-2340

Dr. I. Yates
USDA, ARS, RRC
950 College St. Rd.
Athens, GA 30613 706-546-3523 FAX-3250

D. E. Zimmer
P.O. Box 5677
Athens, GA 30613 706-546-3496 FAX-3401

APPENDIX C

MANAGEMENT UNITS (SURVEY REPORTS)

Chemical Methodologies and Bioassays

* Indicates that the information is also relevant to other research areas and has been included as such.

*

Name: Hamed K. Abbas

Laboratory: Southern Weed Science Laboratory

Address: P.O. Box 350
Stoneville, MS 38776

CRIS #: 6402-22000-012-00D

Telephone #: (601) 686-5313

FAX #: (601) 686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. A method of isolation and purification of the natural products AAL-toxin and its analogues was developed.

2. Many natural products were tested for phytotoxicity on intact plants, duckweed, excised leaves and tissue culture.

3. A method was developed for biosynthesis of hydrolysis products of the fumonisins and AAL-toxin.

4. AAL-toxin, diacetoxyscirpenol and T-2 toxin were shown to have insecticidal activity against tobacco budworm larvae (Heliothis virescens).

5. AAL-toxin was patented (U.S. Patent # 5,256,628) as a herbicide on October 26, 1993.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: The purpose of the overall objective is to identify and evaluate natural products that have potential uses as bioherbicides. This includes: a) development of analogues of AAL-toxin and fumonisins, and b) isolation and identification of new phytotoxins from pathogenic and non-pathogenic fungi. Radicinin (isolated from Alternaria helianthi), tenuazonic acid (isolated from A. alternata), Fusaric acid and moniliformin (isolated from Fusarium spp.) and unidentified phytotoxin appear to have potential in preliminary tests.

2. Significance: Herbicides derived from biological sources often have unique mechanisms of action. They have the potential for replacing current herbicides with more

toxicity or that are losing effectiveness because of development of resistance. Natural products tend to be safer for the environment and more biodegradable than conventional herbicides.

3. Constraints: Fumonisin has been reported to cause mammalian toxicity, which has hindered research on applications for weed control. The research situation including technical help and funding is unstable.

C. Current and Future Cooperators (ARS and Others):

ARS: Stephen O. Duke, Joseph E. Mulrooney, James E. Hanks, Rex N. Paul (Stoneville, MS).

FS: W.T. Shier, T. Krick, C.J. Mirocha (U. Minn.), J. Kuti (Tex. A&I U., Kingsville, TX), G. Kraus (Iowa State U., Ames, IA).

Industry: Luke Lam (LKT Laboratories, Inc., St. Paul, MN).

D. Potential Uses of Research Findings:

Development of fungi and the natural products as herbicides for weed control and determination of mechanism of action of these herbicides. Industry is interested in commercial applications.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

We need cooperation with others in the USDA working on similar projects. it would be helpful to have those working on natural products for weed control at one location. Stability in funding would help to ensure that research objectives could be carried out.

F. Technology Transfer and End-Use Strategies and Opportunities:

AAL-toxin has been patented as a herbicide (U.S. Patent No. 5,256,628 on October 26, 1993). It and its analogues should be of interest to the herbicide industry within the near future. Other products will be marketed to industry as they become available.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Intact plants
Duck Weed
Excised leaves
Tissue Culture
Heliothis virescens

*

Name: Bradley F. Binder

Laboratory: Corn Insects Research Unit

Address: Genetics Laboratory
Iowa State University
Ames, Iowa 50011

CRIS #: 3625-22000-012-00D

Telephone No.: 515-294-6948

FAX #: 515-294-2265

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovery of a family of plants that interrupts insect growth and development by interfering with the endocrine system. Antijuvenile hormones and juvenile hormone mimics were identified and their biological activities studied in the corn earworm, *Helicoverpa zea* and the milkweed bug, *Oncopeltus fasciatus*.

2. Development of a new bioassay to test natural products for their properties as kairomones, allomones, and ovicides against the European corn borer, *Ostrinia nubilalis*.

3. Corn extracts were shown to have activity against European corn borer adults.

B. Research Objectives for Next Five Years (brief description); also fill in attached table using brief description:

(Brief title of each objective)

1. Purpose: Discovery of novel natural products that mediate the behavior and physiology of the European corn borer, corn earworm and the black cutworm.

2. Significance: The pests can be controlled by deterring them from corn or attracting them to other suitable alternate host plants.

3. Constraints(e.g.,regulatory,patent,fiscal,technical, disciplinary, commercialization, etc.): Patents will befiled for novel natural products or synthetic chemicals, based on natural product leads, that mediate the behavior and physiology of corn pests. These compounds may be commercialized in cooperation with industrial companies.

C. Current and Future Cooperators and Their Contributions (ARS and Others):

Richard L. Wilson - USDA/ARS, Plant Introduction Station, Ames, IA -(Entomologist, specialist on new corn accessions).

William B. Showers - Iowa State University, Dept. of Entomology, Ames, IA - (Insect Ecologist).

Carl L. Tipton - Iowa State University, Dept. of Biochemistry, Ames, IA - (Biochemist).

Albert B. DeMilo - USDA/ARS, Insect Chemical Ecology Lab., Beltsville, MD - (Synthetic Chemist).

D. Potential Uses of Research Findings:

Chemicals that mediate the behavior of adults easily can be incorporated into existing integrated pest management programs. For instance, a compound that deters adults from corn could be sprayed on the plants at appropriate times to prevent corn pests from approaching, landing, and ovipositing on these fields. An attractant might be used to draw adults away from corn or other commercially important plants to sites where they have no economic impact.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Chemicals that mediate the behavior of adults could be implemented within existing integrated pest management programs in 5-10 years, once basic laboratory and field studies have been completed.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Most contemporary research programs in Government and Industry focus on control of the immature feeding stages of pests. Many adult pests are extremely mobile and fecund yet highly vulnerable to changes in environment, pathogens, predators and chemicals. Aside from pheromones, few research programs have focused on new ways to control adult movement, orientation, oviposition, and egg development.

G. Chemical Methods Employed:

Conventional extraction and purification protocols for plants will be used. The extracts will be applied to glass plates to test for oviposition activity against the European corn borer.

H. Bioassay methods employed:

A new oviposition bioassay was developed for the European corn borer. This assay manipulates ECB native oviposition behaviors and encourages oviposition only on glass plates. These plates are treated with extracts or pure compounds to test for effects on oviposition.

*

Name: Karen D. Burkhead

Laboratory: National Center for Agricultural Utilization Research

Address: USDA ARS
1815 N. University St.
Peoria, IL 61604

Cris #: 3620-41000-031

Telephone No: 309-681 -6287

FAX #: 309-681-6686

A. <u>Research Accomplishments (up to 5) in Last Five Years:</u>

1. (Present CRIS, 1992-93) Isolated and identified a putative germination inhibitor of wheat from *Pseudomonas fluorescens* 2-79.

2. (Present CRIS, 1992-93) Isolated and identified the antifungal compound pyrrolnitrin from *Pseudomonas cepacia* B37w and demonstrated its antifungal activity against causal agents of potato dry rot.

3. (Present CRIS, 1992-93) Detected from one to eight antifungal compounds from each of twenty bacterial strains by bioautography.

4. (Previous CRIS, 3620-41000-029-02 T, 1990-92) Screened 670 microbial cultures for aryl hydroxylase activities of industrial importance. Identified 47 *Aspergillus* strains and several cultures from other genera able to regiospecifically hydroxylate several model aromatic compounds of industrial interest.

5. (University of Iowa, College of Pharmacy, 1989-90) Isolated and identified aurofusarin from *Fusarium graminearum* and prepared chemical derivatives of this poorly soluble pigment. Reported for the first time C-13 nmr data and biological activity of these naphthoquinone dimers against Gram (+) and Gram (-) bacteria.

<u>B. Research Objectives for Next Five Years (brief description): to isolate and identify antibiotics</u>

1. Purpose: The purpose is to isolate and identify bacterial antifungal compounds which have been detected in potential biocontrol bacteria, using fermentation, extraction, chromatography, and spectrocopic techniques.

2. Significance: a. Knowledge of antibiotic production is

important for formulation of the cultures as biological control products. b. The antifungal compounds themselves may have useful veterinary and human applications, as well as many crop protection possibillities.

3. Constraints: a. One year remains in my current post-doctoral appointment. b. The overall project goal is development of the bacteria as biocontrol agents. The best biocontrol agents are not the best antibiotic producers.

C. <u>Current and Future Cooperators (ARS and Others):</u>

ARS: D. Schisler and P. Slininger (NCAUR, Peoria, IL).

D. <u>Potential Uses of Research Findings:</u>

Microbial cultures may be used for biocontrol of potato dry rot fungi and other crop pathogens.

Antifungal compounds have potential applications in crop protection, veterinary uses, and treatment of human systemic and/or topical infections.

E. <u>Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes</u>

Many agrochemical and pharmaceutical companies demonstrated interest in this project at the 206th ACS National Meeting in Chicago. Aside from the development of cultures as biocontrol agents, there may be separate uses for discovered antifungal compounds. If novel structures are identified within the next year, technology transfer arrangements for new antifungal leads may be readily developed, since the market for antifungal compounds is rapidly growing.

F. <u>Thoughts on Research Needs (not being addressed in other Agencies or at State levels):</u>

Within ARS, natural product leads might be screened for additional activities. For example, antifungal compounds that protect potatoes from dry rot may be useful against other fungal pathogens of other crops, and may have insecticidal activities as well.

For information transfer and technology transfer within ARS, a directory of natural products research activities/leads would be useful. I would be willing to work on the creation of such a directory.

G. <u>Chemical Methods Employed:</u>

Extraction from microbial fermentation, chromatography (flash

column, TLC, HPLC), spectroscopy (MS, NMR). May use GCMS, UV, IR.

H. Bioassay Methods Employed:

Whole potato, bioautography assays for antifungal compounds.

*

Name: Horace G. Cutler

Laboratory: Microbial Products Research Unit

Address: Richard B. Russell Research Center
P.O. Box 5677
Athens, GA 30613

CRIS #: 6612-41000-001-00D

Telephone No.: (706) 546-3378

FAX No.: (706) 546-3250

A. Research Accomplishments in Last Five Years:

1. Isolation and identification of a natural product fungicide. The material is presently in field trials to control *Armillaria* in Kiwifurit and *Pinus radiata* and to control silverleaf in Asian pears and ornamentals. It is biodegradable and safe.

2. Isolation of a bioremediating organism that aerobically breaks down PCPs (under patent).

3. Isolation and identification of (-) harzianopyridone, a fungal natural product with herbicidal properties.

4. Isolation of botcinolide, a novel natural product herbicide.

5. Antimicrobial and plant growth regulating properties of sucrose esters.

B. Research Objectives for Next Five years:

The objective is to isolate, characterize, and utilize biodegradable natural products from microorganisms for agricultural and other uses.

1. Purpose: To isolate, identify and use as herbicides, antimicrobials/antivirals, natural products from microorganisms for practical use as biodegradable chemicals (environmentally benign agents) and/or pharmaceuticals.

2. Significance: To protect the enviroment, to produce quality pesticide free food and to produce value-added products from fermentation.

3. Constraints: Technical - Need NMR facilities and easy access to X-ray crystallography. Need FAB-MS and HRP-MS.

C. Current & Future Cooperators:

- Dr. Robert A. Hill, HortResearch, New Zealand. Microorganisms and field tests. $25,000
- Dr. L. Cheah, Food Research Ltd, New Zealand. Post harvest applications of natural products.
- Dr. H. Rhothitha, HortResearch, New Zealand. Entomology
- Dr. Karst Hoogsteen, Merck Therapeutic Research. X-ray crystallography.
- Dr. Gary Newton, Univ. of Georgia, X-ray crystallography
- Dr. Stephen Cutler, College of Pharmacy, Mercer Univ. Chemical Synthesis
- Dr. John Jacyno, College of Pharmacy, Ohio Northern Univ. Toxicology.

D. Potential Uses of Research Findings:

Biodegradable, environmentally safe agricultural chemicals for use both pre and post harvest to protect crops and products. These will protect consumer health and will protect the export market. In addition, we expect financial profit from these developments.

E. Technology Transfer and End-Use Strategies and Opportunities:

To industry, for development, either before or after the patent process. Some of our materials are already undergoing field trials (Timeframe: now and < 10 years).

F. Thoughts on Research Needs:

There are several natural product sources that are not being tapped because of lack of financial support and, especially, a lack of imagination in spending and <u>obtaining</u> funds.

G. Chemical Methods Employed:

Column chromatography, Prep HPLC, HPLC, TLC, Spinning Plate, UV, FT, R, 1HNMR, ^{13}CNMR, LRP_{MS}, FABMS, X-ray crystallography.

H. Bioassay Methods Employed:

Etiolated wheat coleoptile bioassay; phytotoxicity tests on greenhouse-grown plants; antibacterial and antifungal bioassays; antitumor bioassays (extra-mural).

*

Name: Robert E. Hoagland

Laboratory: USDA/ARS/Southern Weed Science Laboratory

Address: P.O. Box 350
Stoneville, MS 38776

CRIS #: 6402-22000-012-00D

Telephone #: 601/686-5210

FAX #: 601/686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. Developed a hydroponic seedling bioassay system to measure efficacy of pathogens/phytotoxins on weeds.

2. Found phenylalanine ammonia-lyase activity and phenolic metabolism was involved inweed defense against two important weed pathogens, indicating the action of microbial elicitors and/or phytotoxins.

3. Found (with cooperators) a fungal isolate on diseased jimsonweed that produced high amounts of phytotoxin, later identified as fumonisin. Examined the selectivity and action of this compound in various weed and crop plants.

4. Found that several natural products with anti-bacterial activity also have selective phytotoxic activity.

B. Research Objectives for Next Five Years (brief description):

a. Investigate chemical (synthetic and natural product interactions with weed pathogens in order to increase or regulate efficacy and/or host ranges.

b. Examine physiological/biochemical effects of phytotoxins produced by microbes.

1. Purpose: To increase microbial pathogen efficacy, alter or regulate plant host range and to provide basic knowledge about the action of these compounds in plants.

2. Significance: Information will be valuable in the development of new and more environmentally safe natural and/or synthetic herbicides.

3. Constraints: Technically, this research is very long-term.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Cooperators include: ARS, industrial and university scientists.

Contributions of cooperators include: Supply of cultures of potential pathogens for weeds; supply of formulations that promote infectivity; field testing of important laboratory findings.

D. Potential Uses of Research Findings:

Discovery of novel phytotoxins useful as herbicides or herbicide development.

Better understanding of the physiology and biochemistry of pathogen/plant interactions and phytotoxin action.

Regulation manipulation of host range of pathogens for weeds.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Funding stability is needed to meet the research goals.

F. Technology Transfer and End-Use Strategies and Opportunities:

Basic scientific information is published in journals, reviews and/or books. Some novel microbe-plant-chemical interactions that may have patent potential will be transferred to industrial or academic cooperators. This may then lead to product development useful for weed control.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Hydroponic seedling bioassay
Weed and crop plants

Name: Jerry W. King

Laboratory: National Center for Agri. Utilization Res.

Address: 1815 N. University Street
Peoria, IL 61604

CRIS #: 3620-41000-043-00D
3620-42000-007-00D

Telephone #: 309-681-6203

FAX #: 309-681-6686

A. Research Accomplishments (up to 5) in Last Five Years:

1. Development of supercritical fluid processing methods for the extraction of oils, flavors, etc. for agriproducts.
2. Development of analytical SF-based methods to replace the use of organic solvents in the laboratory environment.
3. Development of SFE/immunoassay methods, for "solventless" rapid screening of pesticides in a field or plant environment.
4. Coupling of SFE and SF-cleanup methods on line with analytical characterization instrumentation, such as SFC, GC, and MS.
5. Use of micro-SFE methods for characterizing single seeds, microanalysis of botanical matter.

B. Research Objectives for Next Five Years:

1. Purpose: On CRIS 3620-41000-043, to continue to develop SF-based processing methods for isolating high-value extracts from commodity and speciality agriproducts sources. On CRIS 3620-42000-007, to develop Sf-based cleanup methods for trace analysis of toxicants, applications to nutrient labeling analysis, and to further develop on-line SF-based analytical methodology.

2. Significance: Major thrust of CRIS 3620-42000-007 is total replacement of solvents slated for elimination under EPA mandate scheduled to start in 1995. CRIS 3620-41000-043 significance is to continue development of scaled-up processing methods employing SFs for the extraction and refinement of chemicals from agrisources.

3. Constraints: Extension of above concepts to natural products, including in-field processing and analytical methodology is not manadated under current CRISs. However potential work with interested collaborators may be possible with approval of NPS. Need for collaborators for field extractors would be essential. Monies for construction of a portable extraction unit would have to be secured to test the above

concept. Commercialization of technology has been a problem at NCAUR.

C. Current and Future Cooperators:

Currently, except for occasional gratis extractions, we have no formalized collaborations with cooperators to test the applicability of process or analytical methodology. A brief CRADA with the Forest Products Lab in Madison, WI was dissolved due to lack of cooperation FPL. A possible future CRADA or MOU may be developed with a faculty member at Montana State University. One of the purposes in attending this conference is to secure possible future cooperators and collaborative arrangements, subject to approval of NPS. (W. Martinez)

D. Potential uses of Research Findings:

1. To provide alternative extraction techniques, compatible with new environmental legislation, for use by natural product chemist.
2. To provide new analytical methodology permitting extractions and isolations to be conducting under relatively benign conditions. Such methods could be scaled-up thereby permitting natural products to be isolated under conditions that are not deleterious to their decomposition, etc.
3. Environmental compatibility of SC-CO_2 would allow extractions to be conducted "fieldside", thereby avoiding transport of considerable amounts of biomass to a processing center, with possible degradation of desired products.

E. Technology Transfer and End-Use Strategies and Opportunities:

1. Development of "fieldside: extraction methodology with a suitable collaborator and unded mechanism would accelerate technology transfer to private sector.
2. A related project of potential value would be the "supercritical" spraying of natural and/or chemical pesticides. Success would depend on locating a suitable engineering collaborator or crops spraying specialist. Ecological benefits could be significant.

F. Thoughts on Research Needs:

Use of supercritical fluid technology for natural products processing seems to be largely ignored by the natural products community. Their traditional methods are in jeopardy due to new environmental legislation and this would seem like an ideal time to initiate some collaborative research. NCAUE's supercritical fluid technology effort has been in place since 1980 and offers the advantage of both analytical and processing methodologies in one research group, coupling with a wide range of processing

scale; literally from sub milligram extractions to pilot plant processing of up to 15 lbs. The opportunity to test SF-isolated products by bioassay means is intriguing and may offer the potential of a higher activity isolate, devoid of contaminating solvents, etc.

G. Chemical Methods:

None

H. Bioassay Methods:

None

*

Name: Robert A. Moreau

Laboratory: Plant Science and Technology Research Unit

Address: ERRC/ARS/USDA
600 East Mermaid Lane
Philadelphia, PA 19118

CRIS #: 1935-41000-031 and 1935-41000-036

Telephone #: 215-233-6428

FAX #: 215-233-6559

A. Research Accomplishments (up to 5) in Last Five Years

1.Developed HPLC methodologies for the separation and analysis of lipids from plants and microbes.

2.Developed HPLC methodologies for the separation, analysis, and purification of hopanoids from bacteria.

B. Research Objectives for Next Five Years (brief description).

1. Purpose:
To investigate the possibility that hopanoids may be valuable co-products from the production of fuel ethanol by bacteria such as *Zymomonas mobilis*.

2. Significance:
Hopanoids are a recently-discovered natural product from bacteria and their potential value and usefulness to industry is unknown.

3. Constraints:
It has been difficult to develop new methods to extract and purify sufficient quantities of hopanoids in order to screen them for various biological activities.

C. Current and Future Cooperators (ARS and Others).

ARS: B. Whitaker (BARC)

University: A. Berry (UCDavis).

Industry: We recently negotiated a CRADA with Rohm and Haas Company, to evaluate the hopanoids from *Z. mobilis* for use as potential natural pesticides.

Others: National Cancer Inst. (D. Newman)

D. Potential uses of Research Findings:

Agricultural chemical uses are covered by above-mentioned CRADA. Other uses are being explored.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes);

In addition to the agricultural chemical applications covered by our current CRADA, recent preliminary results indicate that these compounds also have some interesting and unique pharmacological activities, which will provide future technology transfer opportunities.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels);

There is a need to gain a better understanding of the biosynthesis, toxicity, and potential effects of these newly-discovered compounds on the environment.

Our long-range objectives will be based on the screening results obtained during the next year.

G. Chemical Methods Employed

High Performance Liquid Chromatography
Mass Spectroscopy

H. Bioassay Methods Employed

Some of our bioassays have been performed by our industrial partner and some by a commercial contract laboratory.

*

Name: John W. Neal, Jr.

Laboratory: Floral and Nursery Crops Research Unit, USNA

Address: 10300 Baltimore Blvd., Bldg. 470
Beltsville, MD. 20705-2350

CRIS #: 1275-22000-080-00D

Telephone No.: 310-504-9195

FAX #: 301-504-9097

A. Research Accomplishments (up to 5) in Last Five Years:

1. Contributed to a team effort to identify four acylsucrose compounds from *N. gossei* that are biologically active against several soft-bodied species of insects and mites. A patent on these compounds has been awarded to ARS.

2. Contributed to a team effort that isolated biologically active compounds in exocrine secretions from lace bug nymphs that have demonstrated (1) repellent properties against birds, (2) inhibition of prostaglandin synthase activity (in vitro), and (3) a relatively high degree of activity against gram+ bacteria.

3. Developed bioassays to evaluate biorationals against several insect pests and mite species.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: To develop and apply bioassays for the purpose of identifying new biologically active natural products against the sweetpotato whitefly and other pests of ornamental plants.

2. Significance: studies with existing bioassays have led to the identification of natural pesticides from an Australian species of *Nicotiana*. Different genera in other plant families are to be explored for new possibilities.

3. Constraints. By definition, the attention to current research objectives in ARS is basically inflexible and CRIS driven and the creative research environment is constrained and made more rigid through the annual Performance Standards that reflect directly to the CRIS statement. I believe this to be a widely held view among researchers in ARS. Fruitful

ventures by cooperating scientists would be accelerated only if a new ARS policy is promulgated through the ARS Administrator. Otherwise cooperation will remain limited and continue to dominate the No. 1 position for "Needs and Priorities". Finally, the most important need all ARS scientist seek is verbal approval and support from all management levels. The rest will take care of itself.

C. Current and Future Cooperators (ARS and Others):

Cooperators are like moving targets in that they constantly change as programs develop and conclude.

D. Potential Uses of Research Findings:

Insect control in agriculture and urban settings.

E. Technology Transfer and End-USE Strategies and Opportunities:

Developing a CRADA with industry for the formulation and registration of the *Nicotiana* extract for commercial application. Two to three years probable.

ARS should have a specific unit for <u>conducting</u> Tech. Transfer as ARS currently has to <u>write</u> Patents. Being currently involved with such tech transfer activities results in the derailment of research activity. Scientists would rather be involved in research activities with cooperating scientists than being involved with numerous telephone conversations and meetings with inquiring industry representatives. Scientists should be recognized for the discovery and attendant studies with their findings and not be responsible for the commercial exploitation. We are not inherently good at these things.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels).

Recent advances in instrumentation available to chemists has accelerated the ability to both identify and synthesize chemical compounds. Though many plant groups or species were 'screened' or had their products bioassayed 20-30 years ago, it behooves ARS scientists to reexamine the past to now make rapid expansions into the future with new pests control materials. There are many species of plants that have only one insect pest and some have apparently none such as Kudzu. These particularly resistant plants should be examined by teams of scientists to isolate and identify the biologically active compounds as potential pest control agents. The conduct of bioassays against either insects or plants (herbicides) with polar and nonpolar leaf extracts can provide rapid insight for pesticide potential.

G. Chemical Methods employed:

N/A

H. Bioassay Methods Employed:

Methods to assess biological activity requires application with water in both topical and residual activity studies. Tests are conducted on a small scale in greenhouses against a variety of plant pests.

*

Name: George W. Pittarelli, Research Collaborator

Laboratory: Soybean and Alfalfa Research Laboratory

Address: USDA-ARS-PSI
BARC-West, Bldg. 009, Rm. 4
Beltsville, MD 20705

CRIS #: None

Telephone #: (301) 504-6478

FAX #: (301) 504-5867

A. Research Accomplishments in Last Five Years:

A U.S. patent (patent number: 5,260,281) has been awarded to the applicants, Pittarelli et al., for the biological pesticide from *N. gossei* discovered at Beltsville, Maryland.

B. Research Objectives for Next Two Years:

1. Purpose: To report new natural biocompounds, determine the best location to grow *N. gossei*, produce new mutagenic plants, to produce new hybrids that are more suitable for mechanical harvesting, and to make the new adult bioassay technique available to other researchers.

2. Significance: By increasing the yield of sugar esters, we can obtain a larger quantity of crude extract from fewer plants more economically. Using the adult bioassay technique, we are able to measure the mortality of the adult SPWF caused by test compounds and obtain the results in less than two hours.

3. Constraints: It is not easy to obtain these new hybrids, but with the use of tissue culture and growth regulators, it can be done. The only negative point concerning the adult bioassay technique is that it will not demonstrate if the compounds are phytotoxic. This problem can be alleviated by growing several plants and treating the leaves with the compounds to be tested.

C. Current And Future Cooperators:

Arizona: David H. Akey.
Georgia: Orest T. Chortyk, Ray Severson, Mike G. Stephenson.
Maryland: J. George Buta, Jianping Cheng, William R. Lusby,

Claude McKee
North Carolina: Verne A. Sisson.

D. Potential Uses Of Research Findings

1. Determination of the best location to grow *N. gossei* will lead to greater quantities of sugar esters for use as biopesticides. The quantity, quality, and yield of sugar esters can be increased by agronomic manipulation and more effective extraction processes.

2. Genetic alteration of *N. gossei* by mutagenesis appears to produce larger quantities of sugar esters from fewer plants.

3. The hybridization *N. gossei* X *N . tabacum* produces larger plants that are more easily harvested.

4. The adult white fly bioassay for insect mortality requires only two hours in comparison to the white fly nymph bioassay which requires 15 days.

5. Unreported natural compounds from N. spp. are being evaluated. The new bioassay should determine the activities as bioinsecticides, biorepellents, or bioherbicides.

E. Technology Transfer And End-Use Strategies And Opportunities:

The good results of *N. gossei* compounds and any technology developed for its extraction in the future should encourage further investigation of other natural products as biopesticides. The low toxicity of the component parts of the *N. gossei* compounds should be of interest to chemical companies considering new approaches to pest control.

1. The strategies of evaluating the best geographic area along with the agronomic condition for *N. gossei* field production and yield enhancement in the future would help with other spp.

2. The genetic manipulation by mutagens will produce more sugar esters per plant, and may alter other chemicals which are very important for research findings. The mutagenized plant can be registered as a new variety and this technology can be applied to other cultivars spp.

3. The technique to alleviate the lethal seedling problems can help in producing other important hybrids from spp. presenting the same problem.

4. Use of the adult SPWF bioassay shows possibilities for adaptation to our technique against adult SPWF. We can obtain results in two hours. Such technique can be used

with any insecticides.

F. Thoughts On Research Needs:

1. The results from *N. gossei* biopesticide should encourage ARS in establishing a new laboratory at Beltsville, Maryland, which should investigate this important area of research. The new laboratory should work very closely with scientists at Athens, Georgia. In this decade, it is very important to produce new classes of bioinsecticides from different species of crops to protect our environment from toxic residues produced by chemical insecticides.

2. We need better cooperation between the investigators. It is very important to let the investigators know the field results of each test. The financial support should be made available in relation to the type and the amount of research that each investigatorstigator is conducting.

3. In 1994 and 1995 field work, we need to test the following factors: quantity of sugar esters produced by each entry; sugar esters ratio of crude extract/leaf weight; cost of production of crude extract. The entries to be tested are: *N. gossei*; *N. gossei* mutagenic plants; new interspecific hybrids *N. gossei* X *N. tabacum*; new *Nicotiana* spp. very active against SPWF and other insect species.

4. Improvement of the technique for solvent extraction of *Nicotiana* plant material.

G. Chemical Methods Employed:

Purification of biologically active compounds present in *Nicotiana* by solvent partition followed by open column chromatography, TLC, and HPLC. Structure determination was done with EIMS, CIMS, ^{1}H NMR and ^{13}C NMR. Activities of compounds were evaluated at all stages of purification by bioassay.

H. Bioassay Methods Employed:

The adult SPWF and GHWF bioassays were used to detect biological activity. Measurements of dehydration and loss of insect body wax were diagnostic.

Name: Russell Pressey

Laboratory: Richard B. Russell Center

Address: 950 College Station Road
Athens, GA 30613

CRIS #: 6612-43000-006

Telephone #: 706-546-3544

FAX #: 706-546-3250

A. Research Accomplishments (up to 5) in Last Five Years:

1. Isolation and characterization of β-mannanse from tomato fruit. This enzyme appears to be related to fruit softing.

2. Isolation and characterization of uronic acid oxidase, an enzyme that alters pectin and its degradation products.

3. Discovery that oxidized pectic fragments activate the destruction of auxin in plants and thus they may function a natural regulators.

B. Research Objectives for Next Five Years:

1. Purpose: To identify enzymes and cell wall components that may be involved in quality changes in fruits and vegetable.

2. Significance: This information should be useful in improving textural and other properties of fruits and vegetables.

C. Current and Future Cooperators:

1. Dr. Carl Bergman, Complex Carbohydrate Research Center, Athens, GA
2. Dr. Merle Weaver, ARS, Albany, CA
3. Dr. Stanly Kays, UGA

D. Potential Uses of Research Findings:

As critical enzymes are identified in textural changes, purification and characterization studies should lead to methods for altering the levels of these enzymes in fruits and this lead to improved products.

E. Technology Transfer and End-Use Strategies and Opportunities:

Any demonstrated improvement in fruit and vegetable quality should attract the interest of industry because of the quantities and huge values of the products involved.

F. Thoughts on Research Needs:

It will be necessary to apply molecular biology approaches to obtain altered levels of key enzymes in fruit and vegetables.

G. Chemical Methods Employed:

Liquid chromatography, HPLC, Ion chromatography, GC, Electrophoresis, colorimetric methods.

H. Bioassay Methods Employed:

None.

*

Name: David C. Robacker

Laboratory: Subtropical Agricultural Research

Address: 2301 S. International Blvd.

Weslaco, TX 78596

CRIS #: 6204-43000-005-00D

Telephone No.: 210/565-2647

FAX #: 210/565-6652

A. Research Accomplishments (up to 5) in Last Five Years:

1. A chemically defined protein-type attractant for both males and females was developed.

2. A chemically defined attractant for both males and females was developed from host fruit volatiles.

3. Attractiveness of pheromone to both males and females in the field was demonstrated.

4. It was shown that bacteria of numerous species from at least five families are generally attractive to Mexican fruit fly.

5. Differential attractiveness of host-fruit and protein-type baits was linked to adult nutrition and physiology.

B. Research Objectives for Next Five Years (Brief Description):

The objectives are to develop an attractant for a dry trap that is superior to currently used baits and to understand how it works.

1. Purpose: The purpose of the work is to replace the standard McPhail trap containing protein bait currently used to monitor populations of *Anastrepha* fruit flies with a new, more effective dry trap with a chemically defined bait that can emit attractive amounts of chemicals for at least twice as long as currently used traps.

2. Significance: Such a dry trap should be less expensive than the McPhail trap both in actual cost of traps and in manpower needed to run trapping programs. More importantly, more effective traps translate into earlier detection of fly populations and therefore faster and less expensive eradication.

3. Constraints: Current slow-release technology is inadequate. I also need more analytical chemistry support for identification of bacteria-produced attractants and cooperators for field testing of new traps with the various *Anastrepha* species.

C. <u>Current and Future Cooperators (ARS and Others):</u>

ARS: R. Flath (Albany, CA); R. Heath (Gainesville, FL); E. Jang (Hilo, HI)

APHIS: A. Martinez, K. Esau (Mission, TX); D. Chambers (Guatemala City, GUA)

Industry: W. Denton (AgriSense); J. Jenkins (Scentry)

D. <u>Potential Uses of Research Findings:</u>

Discussed above was replacement of the expensive McPhail trap/protein bait trapping system with less expensive dry traps containing long-lasting chemically defined attractants. In addition, this research will increase our understanding of complex relationships of environmental and physiological factors affecting attraction of fruit flies to various baits. Such knowledge could enable users to make informed choices regarding which baits to use under a specific set of conditions and could aid researchers working with other species of Tephritidae to better understand their systems. Finally, this research may introduce new chemical classes as attractants that may function in novel ways.

E. <u>Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):</u>

Development of slow-release technology for these types of chemicals will be conducted by industry during the next year. The attractant technology will be transferred to my industry partner for commercial production by 1995. Large scale pilot testing perhaps in California with CDFA or in Texas with TDA is anticipated for 1996-97.

F. <u>Thoughts on Research Needs (not being addressed in other Agencies or at State levels):</u>

N/A

G. <u>Chemical Methods Employed:</u>
Methods used in my laboratory include various volatile collection techniques, on-column and Grob injection capillary GC, and HPLC.

H. <u>Bioassay Methods Employed:</u>

A rapid laboratory bioassay called a "cage-top" bioassay is used for initial screening of most chemicals. A flight chamber type wind tunnel and local field testing with sterile, released Mexican fruit flies are used to evaluate chemicals and mixtures that seem promising. Field testing in Mexico and Guatemala is employed to evaluate attractants deemed best in local field testing.

*

Scientist: Dr. R. D. Stipanovic
Dr. A. A. Bell
Dr. C. R. Howell

Laboratory: Southern Crops Research Laboratory

Address: Route 5, Box 805
College Station, Texas 77845

CRIS #: 6202-22000-011-00D

Telephone #: 409-260-9232

FAX #: 409-260-9333

A. Research Accomplishments in Last Five Years:

1. Identified critical chemical and anatomical responses that occur in cotton after infection by the wilt pathogen *Verticillium dahliae*.

2. Determined the mode of action of the cotton phytoalexin desoxyhemigossypol.

3. Developed methods to control the biosynthesis of phytotoxins in the biocontrol agent *Gliocladium virens*.

4. Determined the role of secondary metabolite production in the biocontrol efficacy of *Gliocladium virens*.

5. Identified phytoalexins in kenaf.

Research area (3) has resulted in a successful patent.

B. Research Objectives for Next Five Years:

1. Identify and evaluate new biocontrol agents.

a. Purpose: To isolate new biocontrol agents, evaluate their effectiveness, identify new antibiotics, determine biosynthetic pathways, and augment antibiotic production.

b. Significance: This research will provide knowledge and useful materials to improve control of soilborne and seedborne diseases of cotton. These studies will facilitate the developement of new and superior strains of biocontrol agents and formulations that can be used by commercial companies and growers to treat planting seed and potentially field soil.

2. Determine mechanisms of cotton resistance to pathogens.

a. Purpose: To reduce cotton losses to plant pathognes.

b. Significance: A detailed understanding of the mechanism of resistance within *Gossypium* will facilitate improvement of resistance to various pathogens and facilitate cloning useful foreign genes and their transfer into cotton.

C. Current and Future Cooperators

Texas A&M Univ.:	Kamal El-Zik, Dept. of Soil and Crop Sciences C.R. Benedict, Dept. of Biochemistry C. W. Magill, Dept. of Plt. Pathology & Microbiology
Industry:	Gustafson, Inc.

D. Potential Uses of Research Findings

1. New Biocontrol Agents

This research will lead to superior strains of biocontrol agents, allow the development of formulations adapted to commercial use, and provide knowledge of the behavior of biocontrol agents that can be used to manage them effectively under field conditions.

2. Cotton Resistance to Pathogens

The research will identify specific beneficial secondary products within germplasm to allow cloning of genes to improve resistance to cotton pathogens.

E. Technology Transfer and End-Use Strategies and Opportunities:

1. New Biocontrol Agents

The research will be utilized by commercial companies, producers, and scientists concerned with managing cotton health, especially soilborne and seedborne diseases. Information will be useful also to scientists working on plant pathogens in other crops. Strains of *G. Virens* have been field tested. Our goal is to have a product ready for commercial use by 1997.

2. Cotton Resistance to Pathogens

The research will be used by commercial breeders, producers, and scientists concerned with improving cotton health. Our goal is to have the critical genes identified and cloned by 1998.

F. Research Needs:

Bench scientist require freedom to set long-term research goals within the mission of their Unit. While recognizing the need to rapidly transfer laboratory findings to practical applications, the Agency must continue to address long-term, high-risk research.

G. Chemical Methods Employed:

Various chromatographic techniques such as thin layer, open column, low pressure, gas, and high performance liquid chromatography are used to purify and quantitate natural products possessing unique biological activity. Chemical structures are determined using UV/visible, FT-infrared, 1D and 2D-nuclear magnetic resonance, and mass (EI, CI and FAB ionization methods) spectrometry.

H. Bioassay Methods:

Whole plant assays are used to evaluate resistance mechanisms in cotton and related genera. Petri dish assays are used to study efficacy of biocontrol organisms. A 96-well plate is used to determine toxicity of phytoalexins to pathogens and nematodes.

Natural Products for Arthropod Control

* Indicates that the information is also relevant to other research areas and has been included as such.

*

Name: David H. Akey

Laboratory: Western Cotton Research Laboratory

Address: 4135 E. Broadway
Phoenix, AZ 85040

CRIS #: 5344-22-620-005-00D

Telephone: (602) 379-3524

FAX: (602) 379-4509

A. Research Accomplishments (up to 5) in Last Five Years:

1. Successfully incorporated "BT" in IPM System for production of cotton; likewise incorporated soaps and products such as M-PEDE.

2. Successfully used *Beauveria bassiana* as Naturalis L for whitefly control in cotton in the arid southwest of USA.

3. Conducted trials with the insect growth regulator, repellent, antifeedant: NEEM-Azadirachtin (2 companies products) and with numbered compounds of hydrated forms.

B. Research Objectives for Next Five years (brief description; also fill in attached table using brief descriptors):

1. Purpose: Development of information on impact of cultural conventional, and biorational chemical control approaches on pests, natural enemies, role in IPM, and resistance management. Develop "nonchemical" (conventional) alternative control methodology including behavioral chemicals, microbials, and parasites.

2. Significance:
Provide IPM tools, demonstrate use, manage insecticide resistance, and reduce use of convention "hard" pesticides.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):
   a. Efficacy of "biorational" products
   b. Long lag time with EPA registration.
   c. Reluctance of PCA'S, consultants, and producers to achieve knowledge level high enough to really use IPM.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Industry: both small and large companies - develop new products and test them via CRADAS and other agreements. ARS & Universities promote coordinated plans for testing and incorporation of new products in control strategies.

D. Potential Uses of Research Findings:

Produce food and fiber with less adverse environmental consequences and do it more effectively.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Time frames for most products in current test for EPA registration to be achieved is 2-4 years. Opportunities exist for small business to develop "niche" markets for these products. Large companies can develop a line of "environmentally safer" products along with their conventional pesticides. See Table 1 for timeframes.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Issue of proper bioassays for growth regulator agents must be addressed.

G. Chemical Methods Employed:

n/a

H. Bioassay Methods Employed:

Bioassays in green houses are conducted by treating seedling plants in a prescribed fraction of an acre or hectare. Dosages are in AI/Unit area. Other assays are performed by leaf dips into solutions of AI as parts per million. Arthropods (life stage will depend on the product under test) are then exposed to the treated plants. In some cases, the test product may be presented from a source to test fuming action at a specified distance away. Efficacy tests are conducted in field trials with all life stages; the most important efficacy criterion being percent reduction from control.

Name: James E. Baker

Laboratory: Stored-Product Insects R. & D.

Address: 3401 Edwin St.
Savannah, GA 31405

CRIS#: 6605-43000-020-00D

Telephone No.: 912-651-3544

FAX #: 912-651-3500

A. Research Accomplishments (up to 5) in Last Five Years:

1. Screened 104 Eastern soft wheat varieties for α-amylase inhibitor content. Used population models for the rice weevil to determine non-catastrophic (sublethal) effects of wheat varieties with relatively high inhibitor content on subsequent weevil population growth.

2. Evaluated two types of biochemical assays for effectiveness of naturally occurring enzyme inhibitors, such as proteinase inhibitors and α-amylase inhibitors present in plants, as biochemical resistance factors against insects. The order of reactants during preincubation is important in assessing inhibitors effectiveness. Assay methods include: (1) (E + I) + S or (2) (I + S) + E. The 2nd biochemical assay method is thought to give a much more realistic analysis of *in vivo* effectiveness of inhibitors in a feeding insect.

3. Research developed through a Capacity Building Grant with Southern University & A&M College, Baton Rouge, LA included a GC/MS analysis of the content of essential oil (dill oil) of 70 dill cultivar fractions. Several components of the dill oil, including carvone and anethofuran, have been evaluated as grain protectants against several stored grain insects.

4. A CRADA was set up with Nurture Biotech, Missoula, Montana, to evaluate the effectiveness of a slow-release material as a carrier for linalool, a volatile terpenoid with toxicity against several stored product insects.

5. GC analysis of marigold extracts before and after bioassay against the rice weevil. Results were used to estimate breakdown of bioactive thiophenes.

B. Research Objectives for Next Five Years (brief description):

Project: Evaluate phytochemicals and insect-produced

chemicals in the stored grain ecosystem that affect the behavior of parasitoids and predators of stored product insects.

Purpose: Develop a thorough knowledge and complete understanding of the chemical ecology of parasitoid and predator behavior.

Significance: Phytochemical- and host insect chemical-based manipulation of parasitoid and predator behavior to increase their efficiency as biological control organisms for insect pests in stored grain may be possible. With the pending possible loss of methyl bromide as a fumigant, biological control methods for stored product insects may become more important.

Constraints: No restraints at this time.

C. Current and Future Cooperators (ARS and Others):

ARS: J. H. Tumlinson Gainesville, FL, J. L. Zettler, Savannah, GA, D. K. Weaver, Savannah, GA, D. L. Silhacek, Gainesville, FL

University: Owusu Bandele, Southern University, Baton Rouge; Paul Weston, Kentucky State University, Frankfort, KY.

Industry: D. Hynson (Nurture Biotech)

D. Potential Uses of Research Findings:

Develop improved, effective, economical IPM control strategies for insects in the stored grain and stored product ecosystem. Natural product chemicals that affect population development of the pest insects themselves or that improve the effectiveness or efficiency of associated parasitoids and predators can be effective components of the IPM strategy.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

(1) Plant species that produce effective bioactive phytochemicals may possibly developed as a cash crop.

(2) Small companies that rear and market biological control organisms may support and benefit from research that leads to more effective use of these organisms in control strategies.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

(1) Assessment of phytochemical effectiveness against insects should be evaluated for overall effects on population development in addition to direct toxic effects. Use of models to evaluate and predict effects of these chemicals on insect population growth can be an important tool. Sublethal effects of many phytochemicals can significant impact on overall population development.

(2) There is a real need to look for effects of phytochemicals and other natural product chemicals targeted against a specific insect pest on associated parasitoids and predators.

(3) Natural product chemicals are still "chemicals" and should be treated as such. Studies on degradation rates under different abiotic conditions, analytical methodologies, toxicity studies against non-target organisms, etc. will need to be developed.

G. Chemical Methods Employed:

GC/MS

H. Bioassay Methods Employed:

Stored product insects

*

Name: Stephen Beckstrom-Sternberr
James A. Duke

Laboratory: National Germplasma Resource Laboratory

Address: USDA, ARS, B-003 R-227, BARC-West
Beltsville, MD 20705

CRIS: 275-21000-057-00D

Telephone: 301-504-5419

FAX: 301-504-5536

A. Research Accomplishments in Last 5 Years:

Natural Pesticide Database Developed

B. Research Objectives for Next 5 Years:

1. Purpose: To discover or popularize natural pesticide as alternative crop to protectants.
2. Significance: Natural pesticides and medicinal compounds are becoming more popular with consumer and USDA should be more consumer-driven.
3. Constraints: Political, Security. (in foreign locations)

C. Current and Future Cooperators and their Contributions:

Weed Science Laboratory (USDA) (Growing Greenhouse Crops)
CRC Press (published database)

D. Potential Uses of Research Findings:

Crop diversification in the humid tropics.
Reduction in narcotic exports from Peru

E. Technology Transfer and End-Use Strategies:

Trying, with civilian conterparts, also to develop marketable alternative crops, including pesticides, medicine, hypoallergenic rubber, low fat chocolate, antioxidant salad dressings.

F. Thoughts on Research Needs:

Critical evolution would favor synergism between pesticidal compound, in plants. Yes, USDA and Industry goes for the silver bullet.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

*

Name: Kenneth R. Beerwinkle, Agricultural Engineer

Laboratory: Crop Insect Pests Management Research Unit

Address: USDA, ARS, SPA, SCRL
Crop Insect Pests Management Research Unit
Rt. 5, Box 808
College Station, TX 77840

CRIS #: 6202-22000-004-00D

Telephone No.: (409)-260-9351

Fax #: (409)-260-9386

A. Research Accomplishments in Last Five Years:

1. Developed a large helicopter-towed net (5-m^2 inlet) for sampling airborne insects.

2. Characterized seasonal radar and meteorological observations associated with nocturnal insect flight at altitudes up to 900 m AGL in East-Central Texas.

3. Developed an automated, vertical-looking x-band radar system for continually monitoring aerial insect activity.

4. Characterized adult corn earworm emergence in a field corn habitat and described their feeding behavior on dallisgrass ergot honeydew.

5. Developed and olfactometer bioassay system for evaluating the efficacy of various plant volatiles as feeding attractants for adult noctuids.

B. Research Objectives for Next Five Years:

Objectives are to identify volatile chemicals of plant origin that are effective feeding attractants for adult corn earworms and other noctuids and develop synthetic mimics of those chemicals.

1. Purpose: Synthesized mimics of identified plant volatile feeding attractants will be used in formulations of toxic food baits (attracticides) for adult corn earworms and other noctuids.

2. Significance: The use of attracticide baits incorporated into pest management schemes designed to control adult pests at their source of origin has potential for reducing crop insect pest problems with substantially reduced use of synthetic pesticides.

3. Constraints: Specific compositions of natural plant chemical compounds which cause attractancy are complex and difficult to identify.

C. Current and Future Cooperators:

ARS: P. D. Lingren, T. N. Shaver, J. D. Lopez, Jr. (College Station, TX)

D. Potential Uses of Research Findings:

With recent advancements in our understanding of aerial transport mechanisms and moth migration behaviors, coupled with the use of natural pollen analyses to identify migrants, we are rapidly approaching a capability for identifying major source areas for several species of highly-mobile crop insect pests. Successful development and implementation attracticide technologies at the source of origin for these pests has great potential for suppressing their populations over wide areas.

E. Technology Transfer and End-Use Strategies and Opportunities:

Technology transfer will be accomplished through publication of technical manuscripts and use of CRADA's and other strategies as progress warrants.

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

Described by cooperator - T. N. Shaver

H. Bioassay Methods Employed:

1. Laboratory bioassays conducted with dual and multiple (up to six) choice olfactometer apparatus which operate on the Y-junction principle and were developed specifically for quantifying the responses of noctuid adults to chemical volatiles from a variety of sources.

2. Laboratory bioassays with low-speed wind tunnels.

3. Field bioassays using feeding attractant volatile sources as baits on wire-mesh cone traps.

*

Name: Bradley F. Binder

Laboratory: Corn Insects Research Unit

Address: Genetics Laboratory
Iowa State University
Ames, Iowa 50011

CRIS #: 3625-22000-012-00D

Telephone No.: 515-294-6948

FAX #: 515-294-2265

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovery of a family of plants that interrupts insect growth and development by interfering with the endocrine system. Antijuvenile hormones and juvenile hormone mimics were identified and their biological activities studied in the corn earworm, *Helicoverpa zea* and the milkweed bug, *Oncopeltus fasciatus*.

2. Development of a new bioassay to test natural products for their properties as kairomones, allomones, and ovicides against the European corn borer, *Ostrinia nubilalis*.

3. Corn extracts were shown to have activity against European corn borer adults.

B. Research Objectives for Next Five Years (brief description); also fill in attached table using brief description:

(Brief title of each objective)

1. Purpose: Discovery of novel natural products that mediate the behavior and physiology of the European corn borer, corn earworm and the black cutworm.

2. Significance: The pests can be controlled by deterring them from corn or attracting them to other suitable alternate host plants.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.): Patents will be filed for novel natural products or synthetic chemicals, based on natural product leads, that mediate the behavior and physiology of corn pests. These compounds may be

commercialized in cooperation with industrial companies.

C. Current and Future Cooperators and Their Contributions (ARS and Others):

Richard L. Wilson - USDA/ARS, Plant Introduction Station, Ames, IA -(Entomologist, specialist on new corn accessions).

William B. Showers - Iowa State University, Dept. of Entomology, Ames, IA - (Insect Ecologist).

Carl L. Tipton - Iowa State University, Dept. of Biochemistry, Ames, IA - (Biochemist).

Albert B. DeMilo - USDA/ARS, Insect Chemical Ecology Lab., Beltsville, MD - (Synthetic Chemist).

D. Potential Uses of Research Findings:

Chemicals that mediate the behavior of adults easily can be incorporated into existing integrated pest management programs. For instance, a compound that deters adults from corn could be sprayed on the plants at appropriate times to prevent corn pests from approaching, landing, and ovipositing on these fields. An attractant might be used to draw adults away from corn or other commercially important plants to sites where they have no economic impact.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Chemicals that mediate the behavior of adults could be implemented within existing integrated pest management programs in 5-10 years, once basic laboratory and field studies have been completed.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Most contemporary research programs in Government and Industry focus on control of the immature feeding stages of pests. Many adult pests are extremely mobile and fecund yet highly vulnerable to changes in environment, pathogens, predators and chemicals. Aside from pheromones, few research programs have focused on new ways to control adult movement, orientation, oviposition, and egg development.

G. Chemical Methods Employed:

Conventional extraction and purification protocols for plants will be used. The extracts will be applied to glass plates to test for oviposition activity against the European

corn borer.

H. Bioassay methods employed:

A new oviposition bioassay was developed for the European corn borer. This assay manipulates ECB native oviposition behaviors and encourages oviposition only on glass plates. These plates are treated with extracts or pure compounds to test for effects on oviposition.

Name: Wendell E. Burkholder

Laboratory: Stored-Product Insects Res. Unit

Address: USDA, ARS Dept. of Entomology
University of Wisconsin
Madison, WI 53706

CRIS #: 3655-43000-002-00D

Telephone #: 608-262-3795

FAX #: 608-262-3322

A. Research Accomplishments (up to 5) in Last Five Years:

1. Developed grain derived volatiles as insect attractants and pheromone synergists.

2. Developed immunoassay methods for measuring insect contamination of grain/food products (resulted in patent that was licensed to BIOTECT, Austin, TX).

3. Developed monoclonal antibodies to the khapra beetle and two parasitoids.

4. Assisted in joint USAID-USDA project on development of natural products from mint (*Ocimum* spp.) for control of bean insects.

5. Assisted in mapping distribution of the lesser grain borer (*Rhyzopertha dominica*) in Canada by pheromone trapping.

B. Research Objectives for Next Five Years:

The research objectives in our Stored-Product Insects Research Unit are to use ecology, behavior, and biochemistry for biorational management of stored-product pests. Included are studies to isolate and identify volatiles from grain and food products that act as attractants, synergists for pheromones, and toxicants. We also are studying improved immunological detection methods for insects, especially the development of a quick, simple and accurate "dip-stick" method of analysis. The purpose is to improve insect monitoring and detection efficiency.

C. Current and Future Cooperators:

ARS: J. Coffelt (Gainesville, FL)
APHIS: A. Barak (Hoboken, NJ)

Industry/University: Agro-BioTech Corp.; Trece Inc.;

Insects Ltd. (pheromone materials).; Quaker Oats & other milling companies (experimental food materials).; Dr. G. Barrie Kitto, Univ. of Texas; Dr. Melissa Stuart, Kirksville, MO; BIOTECT, Austin, TX (immunoassay studies).

D. Potential Uses of Research Finding:

The results of our research have and are expected to continue to improve insect control. They will increase the quality and quantity of agricultural products in the U.S. and may improve our position in the export market.

E. Technology Transfer and End-Use Strategies and Opportunities:

Several patents have already been issued on our work and we also transferred much data by our publications concerning biology, behavior, pheromone and attractant chemistry, traps etc. We expect further transfer of information with the pheromone, attractant and immunoassay work within one to two years.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State level):

Coordination and cooperation on the natural product insect attractant and repellent research within ARS can be improved. Research centered in one or two large research facilities would be a mistake. Field locations in experiment station facilities often have the opportunity to glean valuable new compounds and ideas that would otherwise be missed.

G. Chemical Methods Employed:

Standard GC, HPLC, GC-MS, immunoassay and related techniques are available.

H. Bioassay Methods Employed:

We use standard and special bioassay methods (pitfall, arena, wind tunnel) that have been published by our laboratory.

*

Name: Laurence D. Chandler

Laboratory: USDA-ARS-IBPMRL

Address: P.O. Box 748
Tifton, GA 31793

CRIS #: 6602-22000-021-00D

Telephone #: 912/387-2326

FAX #: 912/387-2321

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identified new insect growth regulators active against corn earworm, beet armyworm and Fall armyworm. These new insect growth regulators are neural agonists which are currently being developed by Rohm & Haas Co.

2. Developed a system for application of *Heliothis* NPV using chemigation technology in corn. Application of NPV significantly reduced corn earworm numbers coming from spring planted corn. Reduction of corn earworm in corn reduced the number of moths able to infest cotton.

3. Determined that treatment of corn earworm larvae with sublethal amounts of diflubenzuron and RH-5992 results in moth sterility. This information could be important in the development of area wide management programs.

4. Co-developed the product Naturalis (*Beauveria bassiana* mixed with a feeding stimulant and carrier) that is active against boll weevil, cotton fleahopper and sweetpotato whitefly.

5. Evaluated Margosan-O for use against lepidopterous pests of corn, peanut and cotton. The product has significant levels of activity against fall armyworm and corn earworm larvae when applied as either a contact material or ingested by the insect.

B. Research Objectives for Next Five Years (brief description):

Evaluation of insect growth regulators for management of lepidopterous pests of agronomic crops.

1. Purpose: To expand use of IGRs in field situations and

identify methods to integrate their use with othe control measures for implementation into area wide management programs.

2. Significance: IGRs are generally non-toxic to beneficial insects and fit nicely into integrated control programs. Use of IGRs at sublethal levels and in bait formulations may provide new and innovative methods for control of certain insects over large areas. Additionally, they may be able to be combined with some naturally occurring toxicants to improve overall control of the target pest.

3. Constraints: IGRs can pose problems in application. New ways of applying low doses directly to the target organism are needed. Some regulatory problems may exist due to environmental concerns. Need studies targeted at developing combination formulations (i.e. IGR + feeding stimulant + toxicant such as neem or other natural product or biological).

Development of area wide management of corn earworm using NPV applications on corn (In cooperation with J. Hamm).

1. Purpose: To expand NPV applications over larger corn acreage throughout the southern states. Comparison of application methods will be made to determine optimal way of getting the virus to the target.

2. Significance: NPV is a naturally occurring virus that can be easily manipulated for mass application. Development of management strategies for control of corn earworm using NPV in combination with other biological and natural control agents would improve the likelihood for this method to be developed into a large scale management program

3. Constraints: NPV commercial production is limited. An effort is needed to increase interest in this material in the private sector. Additionally, any program targeting field corn will probably needed to be subsidized by a government agency in order to be implemented. Corn economics will not allow growers to pay for the entire cost of a program of this type.

Evaluation of new natural products for use in lepidopterous insect management programs in agronomic crops.

1. Purpose: To identify new natural products (commercially available or from government/University labs) that might be useful in control of beet armyworm, fall armyworm, corn earworm and tobacco budworm.

2. Significance: Any addition to our current knowledge of natural product activity against the named pests will improve our ability to manage the pests. Materials such as limonoids, neem oil and derivatives, and capsaicin are currently being evaluated in our lab and may prove important in future insect control efforts.

3. Constraints: Many natural compounds are not available in large amounts needed for field tests. Additionally, they are very expensive to obtain.

C. Current and Future Cooperators (ARS and Others):

John J. Hamm, USDA-ARS, Tifton, GA - NPV and Bt evaluation
Jim Carpenter, USDA-ARS, Tifton, GA - IGR sterility effects
Harold Sumner, USDA-ARS, Tifton, GA - application technology
Mickey McGuire, USDA-ARS, Peoria, IL - IGR formulations
Baruch Shasha, USDA-ARS, Peoria, IL - IGR formulations
James Wright, USDA-ARS, Weslaco, TX - Naturalis cooperator
Gary Herzog, Univ. GA, Tifton, GA - field evaluations
Edd Harrison, Rohm & Haas Co., Camilla, GA - IGR applications

D. Potential Uses of Research Findings:

The studies outlined here will be useful for growers and the private sector insecticde manufacturer. These studies should also be important in development of area wide management programs for targeted pests (an ARS priority).

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Information will be transferred for growers, other scientists, action agencies and private industry as research results become available. Private industry should have interest in development of new products or combinations of products that are environmentally friendly. Action agency opportunities are dependent upon development of area wide management strategies that may take up five years to develop.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Need studies designed at integrating several control strategies, including natural products/biologicals, into an effective management program. Need additional help from chemists-formulation experts on development of formulations that will remain active for longer periods of time. Need nationwide coordination of programs using the best available biologically friendly control tactics to avoid unnecessary

duplication of studies.

F. Chemical Methods employed:

N/A

G. Bioassays in Use:

Bioassays used consist of topical, leaf dip, diet spread, and diet incorporated methods. In addition a spray table is used to simulate actual field applications. Treated leaves are removed from treated plants and held in cups to evaluate insect mortality.

*

NAME: O. T. Chortyk

LABORATORY: Phytochemical Research Unit

ADDRESS: USDA, ARS, R. B. Russell Agricultural Research Center
P. O. Box 5677, Athens, GA 30613

CRIS #: 6612-21430-001-00D

TELEPHONE #: 706-546-3424

FAX #: 706-546-3454

A. Research Accomplishments (up to 5) in Last Five Years:

Syntheses and characterization of sugar esters and determinations of their biological activities.

B. Research Objectives for Next Five Years (brief description: also fill in attached table using brief descriptors):

Purpose:

(a) To identify biologically active components in plants and to develop synthetic method for their large scale production.

(b) To produce new biologically active compounds.

2. Significance: To develop environmentally safe biorationals.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

C. Current and Future Cooperators and their Contributions (ARS and others):

ARS: R. F. Severson, D. M. Jackson, G. Pittarelli, M. E. Snook, G. Puterka, R. Horvat, D. Barnard, H. Cutler, M. Stephenson

Other: A. Johnson, Clemson; N. Toscano, U. Cal.

D. Potential Users of Research Findings:

Developed biosafe pesticides against sweet potato whiteflies, psylla, aphids, other insects will be most welcomed by farmers and environmentalists.

E. Technology Transfer and End-Use Strategies and Opportunities (include predictive timeframes):

1993--Started cooperative research with industry.

1994--Possible CRADAS with one or more companies

1995-97--large-scale production of sugar esters, field tests, possible commercial product.

F. Thoughts on Research Needs:

None

G. Chemical Methods Employed:

Standard methods of analysis (GC, GC/MS, HPLC) are coupled with purification methods involving column chromatography, chromatotron separations and LH-20 column chromatography.

H. Bioassay Methods Employed:

Insect bioassays on developed compounds are/will be conducted by cooperators, employing insects such as whiteflies and aphids.

Name: Stephen L. Clement

Laboratory: Germplasm Introduction and Testing Research

Address: 59 Johnson Hall
Washington State University
Pullman, WA 99164-6402

Cris #: 5348-21000-010-00D

Telephone No.: 509-335-1502

Fax #: 509-335-6654

A. Research Accomplishments (up to 5) in Last Five Years:

1. First reports of fungal endophytes in grass germplasm held in repositories of the National Plant Germplasm System.

2. First demonstration of grass endophyte conferred resistance to Russian wheat aphid.

3. Demonstration of pea weevil (*Bruchus pisorum*) nectar feeding on pea blossoms and formulation of new hypotheses related to pea weevil-pea interactions.

4. Discovery of antibiosis resistance to pea weevil in wild pea germplasm.

B. Research Objectives for Next Five Years (comments relate only to planned research on natural products to control pests)

Broad Objective: To explore possibility that pea phytochemicals (attractants) mediate the host selection behavior of pea weevil, a strictly monophagous insect that completes its development only on cultivated pea.

1. Purpose: Preliminary studies suggest that pea weevils use pea attractants to find host plant. Can pea phytochemicals be used to lure pea weevil adults to toxic baits?

2. Significance: There is a critical need to develop biologically-based strategies/tools to control pests of minor crops (i.e., field peas), given loss of synthetic insecticides. Moreover, research will identify natural products in plant germplasm that may be important in developing insect control strategies.

3. Constraints: Fiscal and technical. Research will not proceed without plant chemistry research (GC/MS) to complete identification/isolation work. Funding is being sought

through grant proposals to hire plant chemist.

C. Current and Future Cooperators (ARS and Others):

Dr. John Fellman, University of Idaho, Moscow.

D. Potential Uses of Research Findings:

1. Aid in direct control of pea weevil adults by luring them to source-point insecticides in bait stations.
2. Will improve efforts to monitor adult activity cycles so insecticide applications can be timed better.
3. The pea weevil-pea association could prove to be a model system for demonstrating the application of plant attractants to control agricultural pests. This is because the pea weevil enjoys a highly mutualistic association with cultivated pea. *Pisum sativum* is the only host plant of *Bruchus pisorum*.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes).

See preceding comments.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

There is no research effort in ARS or at the State level to characterize head-space volatiles from pea that may mediate the host selection behavior of the pea weevil and other pests. ARS Natural Product Chemists are needed to interact with ARS entomologists at sites and units where chemistry expertise is lacking.

G. Chemical Methods Employed

Procedures for identification of volatiles involve placing flowers, pods, and stems in a closed environment, passing clean air over the plant material and trapping emitted volatiles on Tenax, a porous polyester substrate. Once trapped, volatiles are thermally desorbed and cryofocused onto a 60m x 0.32 mm FSOT DB-WAX capillary column and separated via high-resolution using quadrupole mass spectrometry, matching fragmentation patterns with those in mass spectral libraries or those of authentic compounds.

H. Bioassay Methods Employed:

Record responses of adult female weevils to suspected behavioral modifying phytochemicals from pea in dual-choice olfactometers. Chemicals invoking positive responses would be candidates for field testing.

Name: Thomas A. Coudron

Laboratory: USDA, ARS, Biological Control of Insects

Address: P.O. Box 7629
Research Park Dr., UMC Research Park
Columbia, MO 65205-5001

CRIS #: 3622-22000-012

Telephone No.: 314/875-5361

FAX: 314/875-4261

A. Research Accomplishments (up to 5) in Last Five Years:

1. Isolated a toxin from the venom of the ectoparasitoid *Euplectrus* spp. that arrests the development of lepidopteran larvae without causing paralysis.

2. Found the venom to enhance virosis by the nuclear polyhedrosis baculovirus AcMNPV (e.g. significantly reduces the LC_{50}) and decreases virosis by the baculovirus HzSNPV (e.g. significantly increases the LC_{50}).

3. Characterized the venom effect on storage proteins found in the hemolymph of parasitized insects.

4. Demonstrated that the venom acts directly at the tissue level and not via the central nervous system or endocrine system of the host.

5. Found the venom to be effective on a wide range of factitious hosts, including *Heliothis/Helicoverpa* spp., *Spodoptera* spp., diamondback moth, armyworm, variegated cutworm, green cloverworm, and the cabbage looper.

B. Research Objectives for Next Five Years:

Advance the identification of toxins and nonparalyzing venoms that regulate insect development in ways suitable for incorporation into insect control programs.

1. Purpose: Discover natural products that regulate the development of pest insects.

2. Significance: Environmentally compatible, insect specific, less resistance prone, biorational substitutes for synthetic insecticides.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.): Technical - obtain exotic parasites; establish a rearing program for exotic parasites; isolate sufficient quantities of rare substances for complete chemical characterization; develop bioassays for the various toxins (venoms) that act on different growth stages (e.g. egg, larval, pupal, adult).

C. Current and Future Cooperators and Their Contributions:

ARS - E. Master; B. Schroeder; and R. Wagner (BARC); D. Barry and A. McIntosh (Columbia, MO); S. Ferkovich (Gainesville, FL); and W. Rice (Crowley, LA)

Academia - D. and G. Jones (Univ. Kentucky); B. Puttler (Univ. Missouri); R. Muniappan (Univ. Guam); and T. Bultman (NE St. Univ, Missouri)

D. Potential Uses of Research Findings:

New information on insect venoms and regulation of insect growth will emerge. The increased knowledge of regulatory mechanisms of insect venoms will have an impact on the direction of insect pest control strategies. Such strategies will involve the use of normal and modified venom components that inhibit insect growth. Venom components and their analogs will be administered to pest insects through expression of venom genes inserted into vectors such as baculoviruses, thereby enhancing their biological activity. The research results impact ARS for the success of two ARS Program Strategy areas (Plant Productivity, and Systems Integration), one ARS High Priority Crosscutting Program (Environmentally Compatible Pest Control), and two ARS National Program (*Heliothis* Program, and the Natural Products for Control of Agricultural Pests), and impact at the international level for the control of several agricultural pests now resistant to synthetic pesticides.

E. Technology Transfer and End-Use Strategies and Opportunities:

- Unique toxin(s) that arrests development in lepidopteran larvae.
- Genetically engineering baculovirus that express for arrestant toxins.

F. Thoughts on Research Needs:

Need to "think through" our philosophy of approaching the use of natural products for use in biocides. Questions need to be addressed, such as: is it the best use of our resources and talent to be exploring toxins that are "generalists", i.e. snake venoms, bee venoms, etc.?

G. Chemical Method Employed:

Column chromatography and electrophoresis.

H. Bioassay Methods Employed:

Larval development and host protein profile.

*

Name: Horace G. Cutler

Laboratory: Microbial Products Research Unit

Address: Richard B. Russell Research Center
P.O. Box 5677
Athens, GA 30613

CRIS #: 6612-41000-001-00D

Telephone No.: (706) 546-3378

FAX No.: (706) 546-3250

A. Research Accomplishments in Last Five Years:

1. Isolation and identification of a natural product fungicide. The material is presently in field trials to control *Armillaria* in Kiwifurit and *Pinus radiata* and to control silverleaf in Asian pears and ornamentals. It is biodegradable and safe.

2. Isolation of a bioremediating organism that aerobically breaks down PCPs (under patent).

3. Isolation and identification of (-) harzianopyridone, a fungal natural product with herbicidal properties.

4. Isolation of botcinolide, a novel natural product herbicide.

5. Antimicrobial and plant growth regulating properties of sucrose esters.

B. Research Objectives for Next Five years:

The objective is to isolate, characterize, and utilize biodegradable natural products from microorganisms for agricultural and other uses.

1. Purpose: To isolate, identify and use as herbicides, antimicrobials/antivirals, natural products from microorganisms for practical use as biodegradable chemicals (environmentally benign agents) and/or pharmaceuticals.

2. Significance: To protect the enviroment, to produce quality pesticide free food and to produce value-added products from fermentation.

3. Constraints: Technical - Need NMR facilities and easy access to X-ray crystallography. Need FAB-MS and HRP-MS.

C. Current & Future Cooperators:

- Dr. Robert A. Hill, HortResearch, New Zealand. Microorganisms and field tests. $25,000
- Dr. L. Cheah, Food Research Ltd, New Zealand. Post harvest applications of natural products.
- Dr. H. Rhothitha, HortResearch, New Zealand. Entomology
- Dr. Karst Hoogsteen, Merck Therapeutic Research. X-ray crystallography.
- Dr. Gary Newton, Univ. of Georgia, X-ray crystallography
- Dr. Stephen Cutler, College of Pharmacy, Mercer Univ. Chemical Synthesis
- Dr. John Jacyno, College of Pharmacy, Ohio Northern Univ. Toxicology.

D. Potential Uses of Research Findings:

Biodegradable, environmentally safe agricultural chemicals for use both pre and post harvest to protect crops and products. These will protect consumer health and will protect the export market. In addition, we expect financial profit from these developments.

E. Technology Transfer and End-Use Strategies and Opportunities:

To industry, for development, either before or after the patent process. Some of our materials are already undergoing field trials (Timeframe: now and < 10 years).

F. Thoughts on Research Needs:

There are several natural product sources that are not being tapped because of lack of financial support and, especially, a lack of imagination in spending and <u>obtaining</u> funds.

G. Chemical Methods Employed:

Column chromatography, Prep HPLC, HPLC, TLC, Spinning Plate, UV, FT, R, 1HNMR, ^{13}CNMR, LRP_{MS}, FABMS, X-ray crystallography.

H. Bioassay Methods Employed:

Etiolated wheat coleoptile bioassay; phytotoxicity tests on greenhouse-grown plants; antibacterial and antifungal bioassays; antitumor bioassays (extra-mural).

Name: Frank A. Eischen

Laboratory: TAES, Texas A&M University

Address: 2415 East Highway 83
Weslaco, TX 78596

CRIS: N/A

Telephone: 210-968-5585

FAX: 210-968-0641

A. Research Accomplishments in Last 5 Years:

1. Identified one plant species whose smoke is partiallly effective in controlling the honey bee tracheal mite (*Acarapis woodi*).

B. Research Objectives in Next 5 Years:

1. Purpose: Identify natural products whose smoke is effective in controlling tracheal and *Varroa* mites in honey bees.
2. Significance: These mites are having a devastating effect on the beekeeping industry.
3. Constraints: None at this time.

C. Current and Future Cooperators:

California Beekeepers Association (thru CDFA)

D. Potential Uses of Research Findings:

1. Identification of active compounds could lead in new directions for acaricides effective against these mites.
2. Identification of some plant species have been found to have negative effects on honey bee longevity. These plants should be avoided.

E. Technology Transfer and End-Use Strategies:

1. Possible development of new acaricides for control of *Acarapis woodi* and *Varroa jacobsoni*.

F. Thoughts on Research Needs:

The prospects of getting new control products approved by EPA/FDA is problematic.

G. Chemical Methods Employed:

Identification of natural products.

H. Bioassay Methods Employed:

Tracheal mite (*Acarapis woodii*), Varroa mites (*Varroa jacobsoni*), honey bees.

Name: Carl A. Elliger

Laboratory: Western Regional Research Center

Address: 800 Buchanan Street
Albany, CA 94710

CRIS #: 5325-21220-012-00D

Telephone No.: (510) 559-5821

FAX #: (510) 559-5777

A. Research Accomplishments (up to five) in Last Five Years:

1. Demonstration of the presence of anti-insect chemicals in foliage of *Petunia hybrida*, and structural determination of the most abundant allelochemical (petuniasterone A) as well as a number of related substances (petuniasterone B and -C series).

2. Examination of descrete species of *Petunia* (*P. violacea, P. integrifolia, P. axilaris, P. parodii,* and *P. inflata*) for insect resistance. Analysis of their foliage for the responsible substances and the structural identification of new steroidal types, *i. e.* the petuniolides and the petunianines. Certain of these have at least an order of magnitute higher biological activity than the earlier identified petuniasterone A.

3. Structure-activity relationships of the above steroidal substances were established.

4. Insect inhibitory substances of *Physalis peruviana* were isolated and identified as a new class of steroidal glycoside esters, related to, but distinct from the well known withanolides.

B. Research Objectives for Next Five Years (brief description):

The overall abjective is to increase host plant resistance toward insects by elevating the amount of defensive substances within the plant. Chemical structures must be established, and analytical procedures developed for quantitation of these defensive agents after which plant breeding may be undertaken. This may be done either by traditional interspecific crossing or intergenerically by advanced methods of molecular biology.

1. Purpose: Increase the understanding of the chemical basis of host-plant resistance by isolating and identifying responsible substances that are present in the plant .

2. Significance: Reduce dependence upon applied pesticides. Allow plant breeders to select resistant strains more efficiently by direct analysis of responsible factors.

3. Constraints: Limited knowledge of allelochemicals within plant species distantly related to crop plants. Poorly understood biological requirements for broad plant crosses. Limited bioassay facility.

C. Current and Future Cooperators (ARS and Others):

Lavone Lambert, ARS; Harvey Chan, ARS; Murray Isman, University of British Columbia.

D. Potential Uses of Research Findings:

Identification of specific chemicals as significant resistance factors allows analytical methods to replace time consuming field studies for evaluating insect/pathogen damage. Knowledge of structure-activity may permit the preparation of simple synthetic analogs, possibly with enhanced activity.

E. Technology Transfer and End-Use Strategies:

Essentially, completely successful research would yield new plant varieties that could be marketed as seed. Additionally, certain chemical derivatives of the natural substances could find commercial use.

F. Thoughts on Research Needs (not being addressed in other agencies or at state levels)

At present, host-plant resistance within ARS is rather fragmented, and interest appears to be decreasing. Improved communication would be welcomed.

G. Chemical Methods Emplowed:

All types of extractive and chromatographic procedures, especially preparative HPLC, are used to isolate chemicals associated with the resistance phenomenon. Instrumental methods at our disposal include NMR (400 mHz), both proton and carbon with all modern pulse sequences. Mass spectroscopy with capacity for EI and CI as well as FAB and LSIms is available as is the capability for X-ray crystallographic analysis.

H. Bioassay Methods Employed:

Our group uses a lepidopteran larval growth bioassay which

is based upon incorporation of test substances into artificial diets at a series of concentrations. Larval weight is measured after a standard period. When required, percent pupation and adult emergence is determined.

Name: Robert A. Flath

Labratory: Western Regional Research Ctr.

Address: 800 Buchanan St.
Albany, CA 94710

CRIS #: 5325-22000-010-00D

Telephone #: 510-559-5807

FAX #: 510-559-5777

A. Research Accomplishments (up to 5) in Last Five Years:

1. Part of cooperative effort (with HI researchers) to find effective attractant for Malaysian fruit fly. "Latilure" (a-ionone) use patented.
2. Compared ripeness of papaya fruit with changes in emissions profile from the fruit (headspace GC/MS), to correlate attractiveness to female fruit fly with emission composition.
3. Completed a qualitative and quantitative examination of pheromonal emissions from the male medfly. Examined the effects of fly age and time of day on profile.
4. Isolated and ran bioassays (HI cooperator) on a number of structural analogs of (+)-a-copaene, in effort to relate potency of male medfly lure to structural features.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: To find more effective, useful attractants for the Hawaiian fruit flies.
2. Significance: Development of more potent trap attractants for population monitoring and detection of infestations, and for assessment of the effectiveness of sterile insect mass releases (female attractants).
3. Constraints: a) Cooperative work with HI ARS researchers is complicated by distance. It is necessary to travel more frequently for consultation and joint experimental efforts. Such travel is expensive. b) When relying on headspace trapping for examination of intact host plants or plant parts, it is difficult to collect sufficient material for necessary bioassay evaluations. Fractionation and further bioassay are nearly impossible.

C. Current and Future Cooperators and their Contributions (ARS and others):

Of necessity, any HI fruit fly-related research requires the cooperation of HI ARS researchers, since the flies are under quarantine. These researchers include: Roy Cunningham

(bioactivity of a-copaene analogs; Malaysian fruit fly attractants); Eric Jang (bioassay, host emissions, male medfly pheromone); Nic Liquido (bioassay, wild hosts for HI flies, latilure analogs, other Malaysian fruit fly attractants). Doug Light (WRRC) also a cooperator, on yearly HI working trips.

D. Potential Uses of Research Findings:

Earlier detection and better monitoring of fruit fly infestations will permit suppression efforts to be started at earlier stages of infestation, reducing the need for pesticides and large economic investments to eliminate an outbreak.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Transfer of successful results, namely the identification of more effective (and perhaps more selective) attractants to pheromone/attractant trap esign companies, via patent and/or publication routes.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Better understanding of fruit fly behavior, interaction with environment; Relative importance of semiochemical, visual, humidity cues in responses to the environment; Explanation of the responses of male HI flies to lures ("parapheromones"). Why only males? What is the relationship between such compounds and the responding insect?

G. Chemical Methods Employed:

Headspace trapping, followed by thermal a/or solvent desorption; codistillation with water (vacuum or 1atm.); solvent extraction; spinning band fractional distillation; LC (silica; silver nitrate/silica); HPLC; capillary GC/FID; GC/MS; optical rotation; NMR; FT/IR; derivatization; ref. compound synthesis as req'd.

H. Bioassay Methods Used:

(by cooperators in HI) Flight tunnel screening, field tests, some EAG work.

Name: Donna M. Gibson

Laboratory: Plant Protection Research Unit

Address: U. S. Plant, Soil, and Nutrition Laboratory
Ithaca, NY 14853

CRIS#: 1907-41000-001-00D

Telephone #: 607-255-2359; 255-0271

FAX#: 607-255-2459

A. Research Accomplishments (up to 5) in Last Five Years:

1. Production of taxol, a plant-derived chemotherapeutic, and related taxanes via cell culture.

2. Purification, characterization, and cloning of fungal phytases.

3. Purification and characterization of plant phytases and acid phosphatases.

4. Development of culture media for both plant and fungal systems.

B. Research Objectives for Next Five Years:

1. Purpose: To isolate insecticidal and nematicidal metabolites from entomopathogenic fungi and plant sources. To develop economic bioprocess systems for the production of metabolites.

2. Significance:
a. discovery of novel pesticidal chemistries.
b. development of useful bioprocess systems via cell culture and fermentation.
c. discovery of novel compounds having uses other than as pesticides.

3. Constraints:
Limited toxicological studies conducted to date on compounds; limitations as to natural product chemists to assist in structural identification of active principles.

C. Current and Future Cooperators (ARS and Others):

ARS:R. E. B. Ketchum, S. Krasnoff, B. Brodie, J. Vandenberg, (Ithaca, NY);
R. Moreau (Philadelphia, PA); L. Towill (Ft. Collins, CO)
Cornell: M. Shuler, T. Hirasuna

Colorado State University: J. Linden

D. Potential Uses of Research Findings:

The challenge of this program is to discover naturally-derived toxicants with high target specificity that offer an alternative to conventional pesticides. By using the entomopathogenic fungi and native North American plants which have an ethnobotanical history of use as source material, we have already found several promising candidates for potential use as antifungal, antinsecticidal, or antinematicidal compounds. Screening and identification efforts are directed toward novel compounds with high activity and low non-target effects.

E. Technology Transfer and End-Use Strategies and Opportunities:

Although somewhat premature to predict, some of these novel compounds may be be suitable in bait formulations. Mode of action studies have not been completed yet to ascertain what end-use strategy will be the most advantageous to pursue.

F. Thoughts on Research Needs:

A natural product chemistry service network or interlocation/interdisciplinary research teams would be useful to expedite the research effort through basic to applied fields.

G. Chemical Methods Employed:

Solvent extraction and partitioning; thin layer, solid phase, and column chromatography; high performance liquid chromatography; GC-MS; NMR.

H. Bioassay Methods Employed:

Insect bioassay systems employed include apple maggot fly, house fly, fruit fly, cabbage looper, tobacco budworm, and diamondback moth.

Nematode bioassay systems employed include root lesion nematode and golden cyst nematode.

Name: Patrick Greany

Laboratory: Insect Attractants, Behavior & Basic Biology Research Laboratory

Address: 1700 SW 23rd Dr.
Gainesville, FL 32608

CRIS #: 6615-22000-005

Telephone No.: (904) 374-5763

FAX No.: (904) 374-5781

A. Research Accomplishments in Last Five Years:

1. Demonstrated that the natural plant growth regulator gibberellic acid (GA) can be used to sustain the innate resistance of citrus fruit to attack by tephritid fruit flies.

2. Determined the effect of treatment on fruit peel color and resistance to puncture.

3. Determined the optimal timing of application of GA for sustained fruit fly resistance, and duration of resistance.

4. Determined optimal dose of GA and associated surfactant, as well as optimal type of surfactant, to assure effectiveness of the treatment with the least probability of harm to trees.

5. Determined effect of GA treatment on susceptibility of treated fruit to postharvest susceptibility to decay, and to effect on overall fruit quality after harvest.

6. Determined effect of GA treatment on propensity of fruit to drop spontaneously.

7. Determined the cost-effectiveness of GA treatment through economic modelling.

8. Determined the effect of GA treatment on internal ripening of treated fruit (negligible).

9. Determined effect of application of GA by commercial speed spray equipment.

B. Research Objectives for Next Five Years:

The primary goal of GA-related research in Florida is to evaluate the use of GA for control of the Caribbean fruit

fly (*Anastrepha suspensa*) in large-scale field experiments (of multiple hectares each at a number of locations.

Cooperative research in Mexico is focused on combining GA treatments with malathion bait sprays to assure control of the Mexican fruit fly (*A. ludens*) under even high population pressure.

In Brazil, the main purpose will be to evaluate the overall benefit to orange juice producers associated with the ability to not only reduce susceptibility of oranges to fruit flies, but also to schedule harvests optimally by way of delaying spontaneous fruit abscission.

C. Current & Future Cooperators:

ARS: R. E. McDonald, Wm. Schroeder (Orlando); P. E. Shaw (Winter Haven); Mexico: M. Aluja & A. Birke, Instituto de Ecologia, A.C. (Xalapa, Veracruz); Brazil: A. Malavasi, Univ. Sao Paulo

D. Potential Uses of Research Findings:

Florida: To reduce susceptibility of late-season grapefruit and thereby enable shipment of GA-treated fruit without postharvest disinfestation treatments.

Mexico: To reduce late-season citrus yield losses due to fruit fly infestation.

Brazil: To reduce fruit damage by fruit flies, eliminate several insecticide applications, and to allow better scheduling of harvests.

E. Technology Transfer and End-Use Strategies and Opportunities:

In Florida, it is anticipated that GA treatments will be an accepted adjunct to the Caribbean Fruit Fly-Free Protocol as early as the 1994-95 season. Some growers in Mexico and Brazil have already adopted use of GA for fruit fly control.

F. Thoughts on Research Needs:

Evaluation of the ecological impact of use of GA for fruit fly control in citrus will be needed (e.g., to determine whether other pest populations are affected either positively or negatively).

G. Chemical Methods Employed:

Not performing analytical studies at this time.

H. Bioassay Methods Employed:

Laboratory and field cage exposure of citrus fruit to tephritid fruit flies.

Name: Robert R. Heath

Laboratory: Insect Attractants, Behavior and Basic Biology Research Laboratory

Address: 1700 SW 23rd Dr.
Gainesville, Fl, 32608

Cris #: 6615-22000-008-00D

Telephone #: (904) 374-5735

FAX #: (904) 374-5707

A. Research Accomplishments in Last Five Years:

1. Elucidation of the NMR spectral assignment of lactone pheromone components emitted by Caribbean and Mexican fruit flies.

2. Determination of the equilibrium and stability of Δ^1-pyrroline, a pheromone component of the Mediterranean fruit fly.

3. Determined pheromone production by males of Anastrepha suspensa under natural light cycles in greenhouse studies.

4. Developed a bioassay system for collecting volatiles while simultaneously attracting tephritid fruit flies.

5. Determine the effects of food availability on pheromone production by males of *Anastrepha suspensa*.

6. Evaluated the use of NuLure and ARGO® steepwater (protein baits) formulations for attracting Caribbean fruit flies, *Anastrepha suspensa*.

7. Designed an automated system for use in collecting volatile chemicals released from plants.

8. Determined the effect of gamma radiation on the capability of male medflies to produce pheromone.

9. Determined the effect of pH on the attractiveness of NuLure to Mediterranean and Mexican fruit flies.

10. Developed a "dry" plastic insect trap for monitoring and suppressing populations of the Mediterranean and Mexican fruit flies (Phase 1 - chemical lure and trap design).

B. Research Objectives for Nest Five Years:

The primary goal of our research is to develop system to monitor and suppress insect population using semiochemicals. This includes the isolation, identification, synthesis and formulation of chemical attractants including pheromones, host attractants and other natural products that modify the behavior for a variety of insect pests.

C. Current & Future Cooperators:

Dr. Nancy Epsky, Research Entomologist, (USDA, ARS, Gainesville, FL); Dr. Paul Sharpe, Research Chemist, (USDA, ARS, Gainesville, FL); Dr. Thomas Baker, Professor of Entomology, (Univ. of Iowa); Dr. Jocelyn Millar, Professor of Chemistry, (Univ. of California, Riverside); Dr. David Robacker, Research Entomologist, (USDA, ARS, Weslaco, TX); Dr. Eric Jang, Research Entomologist, (USDA, ARS, Hilo, HI); Dr. Derrel Chambers, Research Entomologist, (USDA, APHIS, Guatemala City, Guatemala); Dr. Gordon Tween, Area Director Central America. (USDA, APHIS-IS), Guatemala City, Guatemala); Dr. Don Linguist, Head of Pest Control, (IAEA). Vienna Austria

D. Potential Uses of Research Findings:

Discovery and improvement of systems to control insect pests will result in decreased dependence on pesticides which are currently used for insect control.

E. Technology Transfer and End-Use Strategies and Opportunities:

Patents for many of the research finding have been or are currently being obtained.

Research that is conducted is often funded by Action Agencies such as APHIS, IEAE, State Agriculture such as California and Florida Departments of Agriculture. The results of our research findings are used by these groups to develop or improve current IPM efforts.

F. Thoughts on Research Needs:

Increased resources and resourceful communication processes would expedite our research.

G. Chemical Methods Employed:

Methods used to isolated and identify insect semiochemical include mass spectroscopy, infrared spectroscopy and nuclear magnetic resonance spectroscopy. Additionally methods such as gas chromatographic and high performance liquid chromatographic are on used on a routine basis.

H. Bioassay Methods Employed:

Laboratory flight tunnels and field cages are employed to observe the behavior of the insects to isolated attractants. After the attractants are obtained and formulated field tests are conducted in the US, Central America and other locations through-out the world.

Name: G. Mark Holman

Laboratory: Food Animal Protection Research Laboratory

Address: Route 5, Box 810, College Station, TX 77845

CRIS #: 6202-32000-003-OOD

Phone: 409/260-9322

FAX: 409/260-9377

A. Research Accomplishments

1. Directed the isolation and structural characterization of fifteen unique neuropeptides from Locust brains/cc complexes

2. Developed method for isolation of neuropeptides from whole-body extracts of small insects (mosquitos, flies). Six new peptides were characterized from mosquitos and three from stable flies.

3. Developed method for isolation of CRF-like diuretic peptides from whole-body extracts of flies and mosquitos. The CRF-DP of the stable fly has been isolated and sequenced.

B. Research Objectives

The focus of the next five years will include: a) diuretic and anti-DH peptides in bloodfeeding diptera, structural identification, stimulation of release, transport and mode of action; b) diuretic and anti-DH peptide receptors. For details, see the 5-year plan; c) toxicology of peptide mimetics.

1. **Purpose:** Obtain structures of the peptides and releasing factors. Provide basic information for structure/activity studies leading to mimetics. Develop receptor assays suitable for screening mimetics.

2. **Significance:** Water and ion balance are critical factors for the well being of insect. Rapid development of mimetics that disrupt those factors could be of great commercial value while being environmentally sound.

3. **Constraints:** None

C. Current and Future Cooperators

* G. M. Coast, Univ. of London, London, U.K.
* L. Schoofs, Katholiecke University, Leuven, Belgium
* D. R. Nässel, Univ. of Stockholm, Stockholm, Sweden
* N. DeDecker, Limburgs University, Diepenbeek, Belgium
* T. Pannabecker, Cornell University, Ithaca, NY
* K. L. Beyenbach, Cornell University, Ithaca, NY
* T. K. Hayes, Texas A&M University, College Station, TX
* S. M. Meola, FAPRL-ARS-USDA, College Station, TX
* R. J. Nachman, FAPRL-ARS-USDA, College Station, TX
* D. L. Bull, FAPRL-ARS-USDA, College Station, TX

D. Potential Uses of Research Findings

Analog studies leading to stable but toxic peptide mimetic compounds suitable for commercial sales.

E. Technology Transfer and End-Use Strategies and Opportunities:

F. Thoughts on Research Needs:

Must be aware of any technological breakthroughs and be prepared to exploit immediately.

Areas of Insect Neurobiology Research which are not being addressed within ARS, but have potential for breakthroughs

* Neuropeptide releasing factors

* Naturally-occurring plant toxins

Insect pest species that might be vulnerable to a neurobiology approach but at present are not being studied with that approach in mind.
Small, but important insects and arthropods. Mosquitos, aphids, ticks, mites.

*

NAME: R. J. Horvat

LABORATORY: Phytochemical Research Unit

ADDRESS: USDA, ARS, R. B. Russell Research Center
P. O. Box 5677
Athens, GA 30613

CRIS #: 6612-21410-005-00D

TELEPHONE #: 706-546-3194

FAX #: 706-546-3454

A. Research Accomplishments (up to 5) in Last Five Years:

1. Volatile constituents from the flowers of 5 *Nicotiana* species have been identified by GC/MS.
2. Volatile compounds of squash leaves and squash trichromes have been identified by GC/MS. These compounds are being currently evaluated for oviposition and attractant stimulants to the female moth (*Diaphania nitidalia*).
3. Blueberry and deerberry volatiles have been identified by GC/MS and will be evaluated for attraction of the adult fly (*Rhagoletis mendax*).

B. Research Objectives for Next Five Years:

The objective is to isolate and chemically identify new biologically active (volatile and semivolatile) compounds from plant parts and flowers of *Nicotiana* species and specific tobacco varieties, with the objective of characterizing environmentally acceptable agrochemicals that may find use as insect attractnats or repellants, insect predator attractants, antimicrobials and natural pesticides.

1. Purpose: The ultimate purpose of this research is to gain knowledge of the compounds responsible for insect (beneficial and pests) attraction to specific plants.
2. Significance: This information could provide plant breeders with the knowledge required to breed plants with minimum levels of these insects pest attractants. Also, plants could be produced to attract beneficial insects.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

None

C. Current and Future Cooperators and their Contributions (ARS and others):

J. K. Peterson and H. Harrison, U. S. Vegetable Laboratory, USDA/ARS, Charleston, SC.

S. D. Pair, South Central Ag. Research Laboratory, Lane, OK

J. A. Payne, Fruit and Tree Nut Research Laboratory, Byron, GA

D. M. Jackson, Crops Laboratory, Oxford, NC

W. S. Schlotzhauer, O. T. Chortyk, H. Cutler, S. Nottingham, USDA/ARS, R. B. Russell Agricultural Research Center, Athens, GA

D. Potential Users of Research Findings:

Plant breeders, entomologists and commercial companies producing biocontrols.

E. Technology Transfer and End-Use Strategies and Opportunities (include predictive timeframes):

This work has not progressed to the point where Technology Transfer, etc. are applicable.

F. Thoughts on Research Needs (not been addressed in other Agencies or at State Levels:

None

G. Chemical Methods Employed:

Leaf volatiles were obtained by hexane and methylene chloride dipping of leaves. These extracts were concentrated and subjected to vacuum steam distillation/hexane extraction in order to remove high molecular weight plant waxes. Hexane concentrates were

analyzed by GC/MS and GC for identification of components.

H. Bioassay Methods Employed:

Wind tunnel experiments are currently being set up for evaluation of individual compounds as insect attractants. Colonies of *Cardiochiles Nigriceps* and *Heliothis Virescens* have been established for use in wind tunnel experiments.

*

Name: Ralph W. Howard

Laboratory: U. S. Grain Marketing Research Laboratory

Address: 1515 College Avenue
Manhattan, KS 66502

CRIS #: 5430-43000-008 and 009

Telephone No.: 913-776-2706

Fax #: 913-776-2792

A. Research Accomplishments (up to 5) in Last Five Years:

1. Purified and characterized stored product insect defense secretions.

2. Showed that several components of these defense secretions are potent prostaglandin synthetase inhibitors (eicosanoid inhibitors) .

3. Elucidated roles of prostaglandins in insect immune response, insect thermoregulation and Malpighian tubule function.

4. Purified and partially characterized a kairomone used by a parasitoid of the Rusty Grain Beetle to locate its host.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop a fundamental understanding of the role of eicosanoids in insect biology, so that these hormones can be manipulated for control purposes.

1. Purpose: Identify insect derived natural products that are potent eicosanoid inhibitors and assess their potential as control agents for stored grain insect pests.

2. Significance: Eicosanoids are insect hormones which have not been manipulated for insect control in the past, but which offer great promise in the future.

3. Constraints: Research area is so new that not enough people

are working in it yet.

C. Current and future Cooperators (ARS and Others):

ARS: Paul Flinn, David Hagstrum, Donovan Johnson, Karl Kramer (Manhattan, KS).

FS: C. A. McDaniel (Gulfport, MS).

Agriculture Canada: Paul Fields, Winnipeg, Canada

University: David Stanley-Samuelson (University of Nebraska); Eric Toolson (University of New Mexico).

D. Potential Uses of Research Findings:

Protection of stored grain from insect pests. Inhibition of eicosanoids in insects should cause them to be severely stressed and increasingly susceptible to environmental stresses such as low temperatures, desiccants, pathogens such as Bt, and to parasitoids.

E. Technology Transfer and End-Use Strategies and Opportunities:

The Agriculture Biotechnology industry can possibly incorporate eicosanoid inhibitors into seed stocks. Several years will probably be required to do so, however.

F. Thoughts on Research needs (Not Being Addressed in Other Agencies or At State Level):

Provide adequate support for non-biotechnological research. We still have a lot to learn about the ecology and behavior of whole organisms and ecosystems.

G. Chemical Methods Employed:

I use HPLC, GC, TLC, and column chromatography; mass spectrometry (EI and CI), NMR, UV, and IR.

H. Bioassay Methods Used:

I use *in vitro* physiological assays to monitor eicosanoid inhibition. I use behavioral bioassays such as trail following for whole organism behavioral research.

Name: D. Michael Jackson

Laboratory: Crops Research Laboratory
Address:P.O. Box 1168,
Oxford, NC 27565

CRIS No.: 6647-22000-004-00D

Telephone No.: 919-693-5151

Fax No.: 919-693-3870

A. Research Accomplishments in Last Five Years:

1. Cuticular components from green tobacco (and *Nicotiana* spp.) were shown to be toxic to tobacco aphids (*Myzus nicotianae*), and LD^{50}'s (lethal dose per adult aphid which kills 50% of the test population) were calculated. Sucrose esters (from various *Nicotiana* spp.), duvatrien-ols, and a combination of duvatrien-ols + *cis*-abienol were the most toxic and caused the greatest reduction in aphid reproduction.

2. Duvane diterpenes and sugar esters from the cuticular exudates of tobacco germplasm were identified as contact ovipositional stimulants for tobacco budworm moths, *Heliothis virescens* (F). *Nicotiana kawakamii* is especially susceptible to oviposition by tobacco budworm moths, and we are identifying the volatile and nonvolatile chemical components responsible for this susceptibility. Components that are highly attractive to tobacco budworm moths might have pest management applications such as trap monitoring, attract-and-kill, or disruption strategies.

3. The attractive properties of volatile components from *Nicotiana tabacum* and *N. kawakamii* are being evaluated by bioassaying the volatiles directly off a GC onto individual receptor cells from tobacco budworm moths (in cooperation with Schlotzhauer and Almaas).

4. Certain *Nicotiana* spp., especially *N. noctiflora*, are highly attractive to *Cardiochiles nigriceps*, a parasitoid of tobacco budworm larvae. Large quantities of leaf material from attractive *Nicotiana* species were extracted with methylene chloride. These extracts are being used for the isolation of volatile components for bioassays against C.

nigriceps. The attractive components from *Nicotiana* species might be used to enhance biological control programs involving *C. nigriceps*.

B. Research Objectives for Next Five Years:

The broad objective is to develop new pest management strategies for use in tobacco and other crops that are safe, efficacious, economically viable, and environmentally benign. The emphasis is on new approaches and new techniques that offer alternatives to chemical insecticides so that overall pesticide usage in tobacco and other crops can be reduced. Specific objectives relative to natural products research are:

1. To investigate the effects that natural chemical and physical characteristics of tobacco and other *Nicotiana* spp. have on the survival, development, and behavior of insect pests and beneficial organisms in the tobacco agroecosystem.

2. To develop novel natural products from *Nicotiana* spp. for use as environmentally safe, biorational pesticides for management of insect pests.

C. Cooperators involved in Natural Products Research:

ARS: R.F. Severson, W.S. Schlotzhauer, O.T. Chortyk, R.J. Horvat, S. Nottingham (Athens, GA); V.A. Sisson (Oxford, NC); M.G. Stephenson (Tifton, GA).

Industry: B. Smeeton (R.J. Reynolds Tobacco Co.).

Foreign: Tor Almaas & Hanna Mustaparta (Univ. Trondheim, Norway).

D. Potential Uses of Research Findings:

1. Novel, biorational insecticides from *Nicotiana* species;
2. Insect behavior-modifying chemicals (attractants, repellents, stimulants) with pest management applications (attracticides, monitoring, disruption, repellency) from *Nicotiana* species.
3. "Feed-stock" chemicals for the production of value-added products from *Nicotiana* species.

E. Technology Transfer:

Patent applications for certain cuticular components from *Nicotiana* species have been made. We have become involved in the development of a multi-state, multi-institutional (including USDA-ARS) Consortium for the Extended Uses of Tobacco. It is envisioned that this consortium would support research and commercial development of such value-added products as pharmaceutical (through genetic engineering), personal care products, foods, industrial enzymes, industrial feed stocks, agri-chemicals (including insecticides), and flavor/fragrances from tobacco.

F. Research Needs:

Money is needed to support large field tests of components isolated from *Nicotiana* species. This would include the growing of large quantities of plant material, extractions, purifications, formulations, and field bioassays.

G. Chemical Methods Employed:

Mass extractions of *Nicotiana* species are made with solvents (methylene chloride, methanol, etc.). Solvents are reduced, and components are separated using column chromatography. Identification of components is done with GC/MS or other techniques.

H. Bioassay Methods Employed:

Ovipositional stimulants are tested in field-cage, greenhouse, and laboratory bioassays by various published techniques. Volatile components are evaluated using flight chambers, and by direct assays on receptor cells. Components with insecticidal properties are tested in topical bioassays in the laboratory, and in small plot field tests. Components thought to affect the feeding behavior of tobacco aphids are tested using electronic feeding monitors.

Name: Michael G. Klein

Laboratory: Horticultural Insects Research Laboratory

Address: USDA, ARS, OARDC
Wooster, OH 44691

CRIS #: 3607-22000-004-00D

Telephone No.: 216-263-3896

Fax #: 216-263-3696

A. Research Accomplishments (up to 5) in Last Five Years:

Soybeans, but not roses or linden, protected from Japanese beetle feeding by Margosan-O. Tests with attractants for northern corn rootworms disclosed that eugenol, cinnamyl alcohol, 4-ethyl-2 methoxypehnol and 4-butyl-2=methoxyphenol are often more attractive in mixtures than exposed separately. Eugenol and geraniol attractive in Florida to *Euphoria sepulchralis*. Japanese beetle floral lures effective for several genera of scarabs in China.

B. Research Objectives for Next Five Years (brief description):

Biologicals for Control of Japanese Beetle and Other Horticultural Insect Pests.

1. Purpose: Identify new attractants for insects, repellents for protecting foliage, and biological insecticides for protecting horticultural crops. Develop new application technologies to maximize efficacy of new natural products.

2. Significance: New pest control strategies are needed to meet demands of cleaner water, human safety, and crop protection.

3. Constraints: New sources of materials and overcoming regulatory obstacles for rapid implementation.

C. Current and future Cooperators (ARS and Others):

J. Harris, W. R. Grace - Provides Margosan formulations
W. Leal, Japan - Provides insect attractants.

D. Potential Uses of Research Findings:

Attractants can be used in regulatory and quarantine work, and may be able to replace insecticides for insect control. Repellants would protect valuable foliage and make traps more useful. Biological materials could replace conventional insecticides.

E. Technology Transfer and End-Use Strategies and Opportunities:

Both the general public and commercial industries are looking for materials and methods to replace conventional insecticides.

F. Thoughts on Research needs (Not Being Addressed in Other Agencies or At State Level):

There is a need to involve research on application technology throughout the process of developing new materials and techniques for controlling pests. We used to get a supply of natural compounds for testing from Dr. T. McGovern, ARS, Beltsville. Since his death, we have not established new contacts and sources of supply.

G. Chemical Methods Employed:

Need the help of chemists to provide materials for use in bioassays.

H. Bioassay Methods Used:

Bioassays will be done under laboratory conditions, in wind tunnel, and more commonly under field conditions against natural populations of Japanese beetles.

Name: Karl J. Kramer

Laboratory: U. S. Grain Marketing Research Laboratory

Address: 1515 College Avenue
Manhattan, KS 66502

CRIS #: 5430-43000-008 and -010

Telephone #: 913-776-2711

FAX #: 913-537-5584

A. Research Accomplishments in Last Five Years:

1. Purified and characterized stored product insect digestive enzymes.

2. Purified and characterized naturally occurring proteinaceous inhibitors of insect digestive enzymes.

3. Identified several antinutritional proteins with insect growth regulating activity.

4. Cloned and characterized the gene for insect chitinase.

5. Transformed a baculovirus with an insect chitinase gene.

B. Research Objectives for Next Five Years:

The broad objective is to develop new physiological, biological and genetic controls for stored-product insect pests.

1. Purpose: Identify proteins that have potential to increase resistance or pesticidal properties of cereal grains and/or biological control agents.

2. Significance: Cereal resistance to insect and microbial attack and the efficacy of biological control agents will be increased through genetic engineering of these proteins.

3. Constraints: Fiscal, personnel and technical limitations.

C. Current and Future Cooperators and their Contributions:

Industry:Thomas Czapla, Pioneer Hi-Bred International- Cooperator on the development of transgenic corn.

University:Subbaratnam Muthukrishnan, Gerald Reeck, Brenda Oppert, Bhuvana Gopalakrishnan and George Liang, Kansas State University- Cooperators on resistance gene cloning and development of transgenic rice and wheat, and transgenic baculoviruses.

D. Potential Uses of Research Findings:

Crop protection against insect and microbial pests.

E. Technology Transfer and End-Use Strategies and Opportunities:

The agricultural biotechnology industry will use findings to enhance host plant resistance to insect pests and fungal pathogens, and to improve biological control agents for insect pests. Biotechnological plants and pesticides should become available within 5-10 years.

F. Thoughts on Research Needs:

Some needs for expertise and personnel can be met through collaborations. Most pressing need is for molecular biologists.

G. Chemical Methods Employed:

High performance liquid chromatography, chemical synthesis, protein purification, recombinant DNA technology, protein and gene squencing, enzyme kinetics, NMR, mass spectrometry.

H. Bioassay Methods Employed:

Oral and injection bioassays using stored product insects, armyworms and hornworms.

Name: Stuart B. Krasnoff

Laboratory: Plant Protection Research Unit

Address: U. S. Plant, Soil, and Nutrition Laboratory
Ithaca, NY 14853

CRIS#: 1907-41000-001-00D

Telephone #: 607-255-7744

FAX#: 607-255-2459

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identification and characterization of phomalactone from *Hirsutella* species.

2. Identification and characterization of viridoxins from Metarhizium flavovinde.

3. Identification and directed biosynthesis of efrapeptins from *Tolypocladium* species.

4. Characterization of insecticidal and antifungal activities of efrapeptins from *Tolypocladium* species.

B. Research Objectives for Next Five Years:

1. Purpose:
   To isolate insecticidal and fungicidal metabolites from entomopathogenic fungi.
2. Significance:
   a. discovery of novel pesticidal chemistries
   b. discovery of extent of toxigenicity in fungal isolates proposed for use as biological control agents
3. Constraints:
   Limited toxicological studies conducted to date on compounds; limitations as to natural product chemists to assist in structural identification of active principles

C. Current and Future Cooperators (ARS and Others):

ARS:D. Gibson, J. Vandenberg, (Ithaca, NY); L. Lacey (Montpelier, France)

D. Potential Uses of Research Findings:

The challenge of this program is to discover naturally-derived toxicants with high target specificity that offer an alternative to conventional insecticides. By using entomopathogenic fungi as biological source material, we have already found several promising candidates from these fungi for use as antifungal and insecticidal compounds. Screening and identification efforts are directed toward novel compounds with high activity and low non-target effects.

E. Technology Transfer and End-Use Strategies and Opportunities:

Although somewhat premature to predict, some of these novel compounds may be be suitable in bait formulations. Mode of action studies have not been completed yet to ascertain what end-use strategy will be the most advantageous to pursue.

F. Thoughts on Research Needs:

A natural product chemistry service network or interlocation/interdisciplinary research teams would be useful to expedite the research effort through basic to applied fields.

G. Chemical Methods Employed:

Solvent extraction and partitioning; thin layer, solid phase, and column chromatography; high performance liquid chromatography; GC-MS, or NRM spectroscopy.

H. Bioassay Methods Employed:

Insect bioassay systems employed include apple maggot fly, house fly, *Drosophila melanogaster*, cabbage looper, and diamondback moth. Fungal bioassay systems include conidial germination inhibition assays against a variety of hypomycetous fungi.

Name: Peter J. Landolt

Laboratory: Insect Attractants, Behavior and Basic Biology Laboratory

Address: USDA, ARS, P. O. Box 14565, Gainesville, FL 32604

CRIS: 6615-22000-009-00D

Telephone No.: (904) 374-5756

FAX No.: (904) 374-5781

A. Research Accomplishments (up to 5) in Last Five Years:

1. A trapping system was developed for female papaya fruit flies based on a combination of a visual fruit model and sex pheromone.

2. A pheromone was discovered, isolated and identified (with R. R. Heath) from male cabbage looper moths that attracts females.

3. It was demonstrated that a material in papaya fruit synergizes sexual attraction in papaya fruit flies and attracts female papaya fruit flies.

4. It was demonstrated that cotton odor synergizes female cabbage looper attraction to male pheromone and stimulates female pheromone release.

5. It was demonstrated that cabbage looper female moths are attracted to the odor of cabbage and cotton and are attracted to damaged cotton.

B. Research Objectives for Next Five Years (brief description:

The principal objective of this program of research involving plant products is to develop a fundamental understanding of host plant effects on sexual behavior of fruit flies and noctuid moths.

1. Purpose: The purpose of the research is to develop novel or better lures and trapping systems for targeted pest species.

2. Significance: The attractants and host plant effects studied are effective against females, which may provide new methods for pest control by removing females from pest populations. Most insect lures are effective only for males.

3. Constraints: In-house limits on space and funding for equipment and personnel prevents accelerating progress. This is partly alleviated by seeking outside funding.

C. Current and Future Cooperators (ARS and Others):

ARS: R. Heath, E. Mitchell, P. Teal (Gainesville, FL), M. Klich (New Orleans, LA).

Israel, Volcani Institute, ARO: E. Dunkelblum and M. Kehat.

Mexico, CEPROBI: M. Camino.

D. Potential Uses of Research Findings:

1. The attractant synergist in papaya fruit odor shall provide a valuable addition to the pheromone-based trapping system developed for female papaya fruit flies. It is expected that this chemical or chemical blend will increase trap efficacy and permit greater flexibility in trap placement. Such a system (fruit model plus pheromone plus host synergist) should be effective as a means of fly pest management to protect papaya fruit. Work on this species has been a model for similar approaches with the medfly and Caribbean fruit fly.

2. Addition of host plant chemicals to the three component pheromone of the male cabbage looper moth may provide a useful lure for female cabbage loopers. Methods for monitoring female moth activity are needed. Information on attractiveness of host odor to ovipositing female moths may also lead to new lures, baits and traps for pest moths.

E. Technology Transfer and End-use Strategies and Opportunities (Include Predictive Timeframes):

1. Information on strategies for fruit fly control has been and will be disseminated through county extension offices and grower groups.

2. When appropriate, cooperating businesses will be sought for development of products and expertise that should be marketed (lures and traps for fruit flies and moths). A patent has been awarded for the papaya fruit fly trap.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

The opportunities for productive research in this area (plant products affecting insect behavior, growth and development, survival) are seemingly unlimited, but the agency has a continuously decreasing number of scientists and technical staff to pursue this type of work. We could be making much more dramatic progress to develop greener alternatives to current pest control practices.

G. Chemical Methods Employed:

Isolation techniques include plant surface washes with solvents and volatile collections (filtering airflow over plants to trap organic chemicals). Identification, synthesis and formulation of chemicals is conducted by collaborators.

H. Bioassay Methods Employed:

Insect attraction responses are assayed in flight tunnels to determine the qualitative nature of flight behaviors. Traps are employed to asses lure attractiveness, both in field cages and in the field.

Name: Juan D. Lopez, Jr.

Laboratory: Crop Insect Pests Management Research Unit

Address: USDA, ARS, SPA, SCRL
Crop Insect Pests Management Research Unit
Rt. 5, Box 808
College Station, TX 77845

CRIS #: 6202-22000-004-00D

Telephone #: 409-260-9351

Fax #: 409-260-9386

A. Research Accomplishments in Last Five Years:

1. Evaluated the feeding response of laboratory-reared and field-collected adult *Helicoverpa zea* to various naturally-occurring sugars from plants to identify effective feeding stimulants. Sucrose is highly stimulatory for feeding; however, the response of the adults varied with the source of the adults (greater in laboratory-reared than in field-collected adults) and the time of evaluation (greater during the evening than during the day).
2. Nectar from three *Gaura* species (Family Onagraceae) elicited a strong feeding response from laboratory-reared and field-collected adult *Helicoverpa zea* and should be useful in the identification of strong feeding stimulants.
3. Malaise traps placed in areas with different densities of ergot-infected dallisgrass indicated that *Helicoverpa zea, Heliothis virescens* and a number of other noctuid pests such as *Spodoptera frugiperda, Pseudaletia unipuncta, Agrotis ipsilon, Spodoptera exigua, Psuedaplusia includes,* and *Trichoplusia ni* were attracted to areas with higher densities of the ergot-infected dallisgrass. The adults were feeding on honey-dew produced by the ergot-infected dallisgrass.

B. Research Objectives for Next Five Years:

Objective: Bioassay and identify feeding stimulants from plants or sucking insects for adult *Helicoverpa zea* and other noctuids and formulate feeding attractants/stimulants with biologically active materials for practical field use

to effect control of the adults.

1. Purpose: Strong feeding stimulants are needed to develop adult control technology.

2. Significance: Control efforts directed at the adults using feeding attractants/stimulants in combination with biologically-active materials should result in the use of reduced amounts of less toxic materials that should be more environmentally compatible and safer for use in food production.

3. Constraints: Primarily technical in that the attractants/stimulants have to be identified and formulated with biologically active materials that will effect control in the field by feeding or contact.

C. Current and Future Cooperators:

ARS: P. D. Lingren, T. N. Shaver, K. R. Beerwinkle (College Station, TX)

D. Potential Uses of Research Findings:

If the adult control technology is successfully applied against *H. zea*, it potentially could be used to control other important insect pests such as tobacco budworm, *Heliothis virescens;* black cutworm, *Agrotis ipsilon;* fall armyworm, *Spodoptera frugiperda;* true armyworm, *Pseudaletia unipuncta;* beet armyworm, *Spodoptera exigua*; cabbage looper, *Trichoplusia ni;* soybean looper, *Pseudaplusia includes,* and numerous other moth pest species.

E. Technology Transfer and End-Use Strategies and Opportunities:

There should be considerable opportunity for technology transfer because ofthe very high losses and control costs due to noctuid insect species. A CRADA will be developed as soon as the technology is sufficiently developed to have a good assessment of its marketing potential.

F. Thoughts on Research Needs:

None

G. Chemical Methods Employed:

HPLC and GC

H. Bioassay Methods Employed:

Bioassays initially involve the proboscis extension response of the adults followed by determination of feeding time and volume to evaluate gustatory response. Adult responses in the field to different formulations containing dyes will be determined by spectrophototometric analysis of samples collected from the crop of the adults.

Name: Everett R. Mitchell

Laboratory: Insect Attractants, Behavior and Basic Biology Laboratory

Address: USDA, ARS, P. O. Box 14565,
1700 SW 23rd Drive
Gainesville, FL 32604

CRIS: 6615-22000-007-00D

Telephone No.: (904) 374-5710

FAX No.: (904) 374-5781

A. Research Accomplishments (up to 5) in Last Five Years:

1. A flight tunnel was developed to assay behavioral responses of tobacco budworm females, *Heliothis virescens*, to plant volatiles.

2. Tobacco budworm females were attracted to volatiles from extracts of cotton, susceptible tobacco, and a wild host, *Desmodium tortuosum*. In choice tests, females flying upwind responded selectively to a variety of susceptible tobacco over a resistant variety.

3. Females arriving at the dispenser emitting plant volatiles exhibited a variety of behaviors including oviposition probes and often extended of the proboscis in a feeding posture.

B. Research Objectives for Next Five Years (brief description):

The objective is to develop attractants for tobacco budworm females.

1. Purpose: The ultimate goal of this research is to develop lures that can be used in traps to monitor populations and as attracticide baits in area-wide management programs.

2. Significance: Female-produced sex pheromones that attract males generally have been ineffective as predictive tools for monitoring insect pests; and the tobacco budworm has

been no exception. An effective attractant for female tobacco budworm moths would greatly enhance the possibility of using trap captures as a predictive tool in pest management schemes and possibly provide a direct control method via attractive bait formulations laced with an acceptable insecticide.

3. Constraints. A bioassay is in place. The primary constraint is the lack of chemical expertise to identify attractive chemicals.

C. Current and Future Cooperators (ARS and Others):

ARS: Fred C. Tingle (IAB&BBRL, Gainesville, FL)

D. Potential Uses of Research Findings:

ARS and cooperating federal, state, and local governments plan to initiate area-wide management of several insect pests over the next few years. The tobacco budworm is a prime target pest for one of these programs. An attractant that would lure the female tobacco budworm to traps or bait stations would be especially beneficial to such a program. Captures of female moths would be helpful in monitoring population shifts; determining the efficacy of such programs as mating disruption and sterile insect releases; and possibly as a direct control component via the use of a combination of attractants with effective insecticides.

E. Technology Transfer and End-User Strategies and Opportunities:

Female attractants would be used by action agencies in area-wide management programs as noted in D and by growers and crop consultants to time pesticide applications at the farm level.

F. Thoughts on Research Needs:

There is an urgent need to link the chemical expertise with the current entomological effort to identify the active materials extracted from host plants such as cotton, tobacco, etc. This liaison would work best if chemistry and behavioral work could be conducted at the same facility.

G. Chemical Methods Employed:

To be determined.

H. Bioassay Methods Employed:

The initial identification of possible attractants would be done in a laboratory wind tunnel. Methods for handling the insects and environmental parameters for wind tunnel bioassays have been worked out. The final stage would be field testing of candidate formulations of attractant chemicals identified from the laboratory assays.

Name: Ronald J. Nachman

Laboratory: Food Animal Protection Research Laboratory

Address: Route 5, Box 810, College Station, TX 77845

CRIS #: 6202-32000-003-OOD

Phone: (409) 260-9315

FAX #: (409) 260-9377

A. Research Accomplishments

1. Structural elucidation of the sulfakinin insect neuropeptide family, first sulfated peptide identified from an invertebrate source.

2. Developed first active pseudopeptide analog of an insect neuropeptide family.

3. Developed first non-peptide mimetic agonist of an insect neuropeptide family.

4. First characterization of the active conformation of an insect neuropeptide family (pyrokinin/PBAN family) required to elicit hindgut/oviduct myotropic and pheromonotropic activity.

5. Characterization of the active conformation of the achetakinin/leucokinin insect neuropeptide family.

B. Research Objectives

1. Structure-activity and active conformation studies of established and newly-discovered insect peptide families.

2. Synthesize and evaluate pseudopeptide modifications of established and new insect neuropeptides.

3. Develop antibodies to active, restricted conformation analogs as receptor models.

4. Utilize synthetic turn-mimetic systems to prepare neuropeptide mimics.

5. Non-peptide mimetic development (with computer-aided screening of 3-dimensional organic chemical data banks).

C. Current and Future Cooperators

* G.M. Holman, FAPRL/ARS, College Station, TX
* R. Beier, FAPRL/ARS, College Station, TX
* F. Clottens, FAPRL/ARS, College Station, TX
* V. Roberts, Scripps Research Institute, La Jolla, CA
* M. Horiharan, Scripps Research Institute, La Jolla, CA
* J. Dyson, Scripps Research Institute, La Jolla, CA
* M. Khan, Univ. of Washington, Seattle, WA
* G. Coast, Univ. of London, London, United Kingdom
* A. Suzuki, Tokyo University, Tokyo, Japan
* Dr. P. Yamamoto, Mitsubishi-Kasei Life Science Inst., Tokyo, Japan
* Prof. O. Yamashita, Nagoya Univ., Nagoya, Japan
* Dr. L. Sreng, CNRS, Marseille, France
* Dr. L. Schoofs, Univ. of Leuven, Leuven, Belgium
* Prof. A. DeLoof, Univ. of Leuven, Leuven, Belgium
* Dr. T. Hayes, Texas A&M Univ., College Station, TX
* Dr. D. Konopinska, Wroclaw Univ., Wroclaw, Poland

D. Potential Uses of Research Findings

The research goal is to develop pseudopeptide and non-peptide agonists/antagonists capable of disrupting the internal balance of insects that are maintained by neuropeptides. Such analogs are potential candidates for selective, environmentally-safe insect pest management agents of the future. Such agents may be suitable for delivery by traditional methods.

E. Technology Transfer and End-Use Opportunities

F. Thoughts on Research Needs

Receptor isolation for direct analog binding studies; improved bioassay systems; mimetic agonists/antagonists; and eventually, delivery systems for mimetics.

G. Chemical Methods Employed

Development of pseudopeptide and nonpeptide mimetic analogs of insect neuropeptides (the natural products) involves use

of a varied repertoire of synthetic chemical reagents and strategies.

H. Bioassay Methods Employed

Diuretic visceral muscle contraction, pheromonotropic, diapause induction and cuticular melanization bioassays will be utilized.

*

Name: John W. Neal, Jr.

Laboratory: Floral and Nursery Crops Research Unit, USNA

Address: 10300 Baltimore Blvd., Bldg. 470
Beltsville, MD. 20705-2350

CRIS #: 1275-22000-080-00D

Telephone No.: 310-504-9195

FAX #: 301-504-9097

A. Research Accomplishments (up to 5) in Last Five Years:

1. Contributed to a team effort to identify four acylsucrose compounds from *N. gossei* that are biologically active against several soft-bodied species of insects and mites. A patent on these compounds has been awarded to ARS.

2. Contributed to a team effort that isolated biologically active compounds in exocrine secretions from lace bug nymphs that have demonstrated (1) repellent properties against birds, (2) inhibition of prostaglandin synthase activity (in vitro), and (3) a relatively high degree of activity against gram+ bacteria.

3. Developed bioassays to evaluate biorationals against several insect pests and mite species.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: To develop and apply bioassays for the purpose of identifying new biologically active natural products against the sweetpotato whitefly and other pests of ornamental plants.

2. Significance: studies with existing bioassays have led to the identification of natural pesticides from an Australian species of *Nicotiana*. Different genera in other plant families are to be explored for new possibilities.

3. Constraints. By definition, the attention to current

research objectives in ARS is basically inflexible and CRIS driven and the creative research environment is constrained and made more rigid through the annual Performance Standards that reflect directly to the CRIS statement. I believe this to be a widely held view among researchers in ARS. Fruitful ventures by cooperating scientists would be excellerated only if a new ARS policy is promulgated through the ARS Administrator. Otherwise cooperation will remain limited and continue to dominate the No. 1 position for "Needs and Priorities". Finally, the most important need all ARS scientist seek is verbal approval and support from all management levels. The rest will take care of itself.

C. Current and Future Cooperators (ARS and Others):

Cooperators are like moving targets in that they constantly change as programs develop and conclude.

D. Potential Uses of Research Findings:

Insect control in agriculture and urban settings.

E. Technology Transfer and End-USE Strategies and Opportunities:

Developing a CRADA with industry for the formulation and registration of the *Nicotiana* extract for commercial application. Two to three years probable.

ARS should have a specific unit for <u>conducting</u> Tech. Transfer as ARS currently has to <u>write</u> Patents. Being currently involved with such tech transfer activities results in the derailment of research activity. Scientists would rather be involved in research activities with cooperating scientists than being involved with numerous telephone conversations and meetings with inquiring industry representatives. Scientists should be recognized for the discovery and attendant studies with their findings and not be responsible for the commercial exploitation. We are not inherently good at these things.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels).

Recent advances in instrumentation available to chemists has accelerated the ability to both identify and synthesize

chemical compounds. Though many plant groups or species were 'screened' or had their products bioassayed 20-30 years ago, it behooves ARS scientists to reexamine the past to now make rapid expansions into the future with new pests control materials. There are many species of plants that have only one insect pest and some have apparently none such as Kudzu. These particularly resistant plants should be examined by teams of scientists to isolate and identify the biologically active compounds as potential pest control agents. The conduct of bioassays against either insects or plants (herbicides) with polar and nonpolar leaf extracts can provide rapid insight for pesticide potential.

G. Chemical Methods employed:

N/A

H. Bioassay Methods Employed:

Methods to assess biological activity requires application with water in both topical and residual activity studies. Tests are conducted on a small scale in greenhouses against a variety of plant pests.

*

Name: William C. Nettles, Jr.

Laboratory: Subtropical Agricultural Research

Address: 2413 East Highway 83
Weslaco, TX 78596

CRIS #: 6204-22000-006-00D
6204-22000-006-01T

Telephone No.: 210-969-4868

FAX #: 210-969-4888

A. Research Accomplishments (up to 5) in Last Five Years:

1. Confidential because of pending patents and CRADA funded by CIBA-Geigy. Results are centered around development of artificial diets and identification of growth factors and ovipositional stimulants from host insects.

B. Research Objectives for Next Five years (brief description):

1. Purpose: Mass production of parasitic insects for control of pest insects, specifically identify ovipositional stimulants, growth factors, and possibly antibiotics.

2. Significance: Provide more effective control of pest insects without the use of chemical insecticides.

3. Constraints: Technical, fiscal.

C. Current and Future Cooperators (ARS and Otherwise):

CIBA-Geigy: A total of almost $400,000 over the last 5 years.

Several other companies likely would be involved except for CIBA-Geigy support.

D. Potential Uses of Research findings:

Large scale automated in vitro production of *Trichogramma* spp. (egg parasites) and tachinids (parasitic flies that attack pest larvae such as *Heliothis*, *Helicoverpa*, gypsy moth, etc.) for field release and control of pest insects.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

CIBA-Geigy has the right of first refusal and likely will purchase licenses to use the several patents that will be obtained. Time: 2-5 years.

F. Thoughts on Research Needs (not being addressed in other agencies or at State levels):

Much greater chemical and engineering expertise is needed. Work needs to be located in a less isolated area where there are better opportunities for greatly expanded interactions with scientists from a broad range of disciplines (analytical chemistry, biochemistry, agricultural, mechanical and chemical engineering, microbiology).

G. Chemicals Methods Employed:

Liquid and HPL

H. Bioassay Methods Employed:

Oviposition into artificial eggs. Growth and development of purified extracts added to artificial diets.

*

Name: Joseph Peterson and Howard Harrison

Laboratory: USDA-ARS, U. S. Vegetable Laboratory

Address: 2875 Savannah Highway, Charleston, SC 29414

CRIS #: 6659-22000-005 and 6659 22000 006

Telephone: (803) 556-0840

Fax: (803) 763-7013

A. Research Accomplishments in Last Five Years

1. Showed presence of chemicals in sweetpotato leaves and tubers that confer resistance to insects.

2. Isolated host plant attractants and oviposition stimulants from squash leaves for the pickleworm, *Diaphania nitidalis*.

B. Research Objectives for Next Five Years

Bioassay guided isolation of natural compounds from vegetable germplasm which confer resistance to insects, primarily involving glycosidic cyclic macroesters, alkaloids, non-protein amino acids and terpenoids.

1. Purpose: Assess role of natural compounds in germplasm of vegetables and closely related species. Develop efficient analytical and bioassay methods to show presence and levels of these compounds.

2. Significance: Knowledge gained may lead to (a) techniques to rapidly identify resistant germplasm for breeding programs; (b) release information to scientists interested in gene transfer and (c) useful natural products either extracted or synthesized for use in insect control strategies.

3. Constraints: Limited interdisciplinary assistance from entomologists in bioassay work, and limited availability of chemical analytical expertise for the purpose of structure elucidation.

C. Cooperators:

ARS: Robert Horvat, Athens; James Porter, Athens; Bill Lusby, Beltsville; Kent Elsey, Charleston, SC. University: Merle Shepard, Clemson University, Charleston, SC.

D. Potential Uses of Research Findings:

a. Use of insect resistance factors to quickly identify (chemical analytically) resistant genotypes and accelerate breeding of vegetables for insect resistance.

b. Identify candidates for intraspecific or interspecific crossing or artificial gene transfer.

c. Identify candidate compounds which play a role in insect resistance and may be synthesized or extracted for use as natural pesticides.

E. Technology Transfer and End Use Strategies and Opportunities

a. Release of insect resistant vegetable cultivars
b. Extraction or synthesis of natural insecticides
c. Transfer of genes into efficient organisms for the production of natural insecticides.

F. Thoughts on Research Needs:

ARS has limited availability of expertise and equipment with respect to NMR and X-ray analyses for the identification of complex natural compounds.

G. Chemical Methods Employed

- Liquid and solid phase extractions
- Capillary gas chromatography
- Low pressure prep. and semi-prep. column chromatography
- Thin-layer chromatography with chromo- and fluorogenic visulatization (any phase)
- HPLC with diode array and mass detector (evaporative light scattering detector) with column switching and recycling capabilities. Use normal and reverse phases, ion-exchange and small molecule gel permeation chromatography (Analytical setup)
- HPLC semiprep and prep set-up Hydrolyses, esterifications

H. Bioassay Methods Employed

Visitations and oviposition on artificial sites treated with attractants and/or oviposition stimulants. Organism: Pickleworm moth, *Diaphania nitidalis*.

Feeding of intact plant parts. Organisms: banded cucumber beetle, *Diabrotica balteata*; spotted cucumber beetle, D. *undecimpunctata howardi*.

Growth and survival of larvae on artificial diets containing isolated compounds. Organisms: Diamondback moth, *Plutella xylostella*; Fall armyworm, *Spodoptera frugiperda*.

*

Name: George W. Pittarelli, Research Collaborator

Laboratory: Soybean and Alfalfa Research Laboratory

Address: USDA-ARS-PSI
BARC-West, Bldg. 009, Rm. 4
Beltsville, MD 20705

CRIS #: None

Telephone #: (301) 504-6478

FAX #: (301) 504-5867

A. Research Accomplishments in Last Five Years:

A U.S. patent (patent number: 5,260,281) has been awarded to the applicants, Pittarelli et al., for the biological pesticide from *N. gossei* discovered at Beltsville, Maryland.

B. Research Objectives for Next Two Years:

1. Purpose: To report new natural biocompounds, determine the best location to grow *N. gossei*, produce new mutagenic plants, to produce new hybrids that are more suitable for mechanical harvesting, and to make the new adult bioassay technique available to other researchers.
2. Significance: By increasing the yield of sugar esters, we can obtain a larger quantity of crude extract from fewer plants more economically. Using the adult bioassay technique, we are able to measure the mortality of the adult SPWF caused by test compounds and obtain the results in less than two hours.
3. Constraints: It is not easy to obtain these new hybrids, but with the use of tissue culture and growth regulators, it can be done. The only negative point concerning the adult bioassay technique is that it will not demonstrate if the compounds are phytotoxic. This problem can be alleviated by growing several plants and treating the leaves with the compounds to be tested.

C. Current And Future Cooperators:

Arizona: David H. Akey.
Georgia: Orest T. Chortyk, Ray Severson, Mike G. Stephenson.
Maryland: J. George Buta, Jianping Cheng, William R. Lusby, Claude McKee
North Carolina: Verne A. Sisson.

D. Potential Uses Of Research Findings

1. Determination of the best location to grow *N. gossei* will lead to greater quantities of sugar esters for use as biopesticides. The quantity, quality, and yield of sugar esters can be increased by agronomic manipulation and more effective extraction processes.
2. Genetic alteration of *N. gossei* by mutagenesis appears to produce larger quantities of sugar esters from fewer plants.
3. The hybridization *N. gossei* X *N . tabacum* produces larger plants that are more easily harvested.
4. The adult white fly bioassay for insect mortality requires only two hours in comparison to the white fly nymph bioassay which requires 15 days.
5. Unreported natural compounds from N. spp. are being evaluated. The new bioassay should determine the activities as bioinsecticides, biorepellents, or bioherbicides.

E. Technology Transfer And End-Use Strategies And Opportunities:

The good results of *N. gossei* compounds and any technology developed for its extraction in the future should encourage further investigation of other natural products as biopesticides. The low toxicity of the component parts of the *N. gossei* compounds should be of interest to chemical companies considering new approaches to pest control.

1. The strategies of evaluating the best geographic area along with the agronomic condition for *N. gossei* field production and yield enhancement in the future would help with other spp.
2. The genetic manipulation by mutagens will produce more sugar esters per plant, and may alter other chemicals which are very important for research findings. The mutagenized plant can be registered as a new variety and this technology can be applied to other cultivars spp.
3. The technique to alleviate the lethal seedling problems can help in producing other important hybrids from spp. presenting the same problem.

4. Use of the adult SPWF bioassay shows possibilities for adaptation to our technique against adult SPWF. We can obtain results in two hours. Such technique can be used with any insecticides.

F. Thoughts On Research Needs:

1. The results from *N. gossei* biopesticide should encourage ARS in establishing a new laboratory at Beltsville, Maryland, which should investigate this important area of research. The new laboratory should work very closely with scientists at Athens, Georgia. In this decade, it is very important to produce new classes of bioinsecticides from different species of crops to protect our environment from toxic residues produced by chemical insecticides.
2. We need better cooperation between the investigators. It is very important to let the investigators know the field results of each test. The financial support should be made available in relation to the type and the amount of research that each investigatorstigator is conducting.
3. In 1994 and 1995 field work, we need to test the following factors: quantity of sugar esters produced by each entry; sugar esters ratio of crude extract/leaf weight; cost of production of crude extract. The entries to be tested are: *N. gossei*; *N. gossei* mutagenic plants; new interspecific hybrids *N. gossei* X *N. tabacum*; new *Nicotiana* spp. very active against SPWF and other insect species.
4. Improvement of the technique for solvent extraction of *Nicotiana* plant material.

G. Chemical Methods Employed:

Purification of biologically active compounds present in *Nicotiana* by solvent partition followed by open column chromatography, TLC, and HPLC. Structure determination was done with EIMS, CIMS, ^{1}H NMR and ^{13}C NMR. Activities of compounds were evaluated at all stages of purification by bioassay.

H. Bioassay Methods Employed:

The adult SPWF and GHWF bioassays were used to detect biological activity. Measurements of dehydration and loss of insect body wax were diagnostic.

Name: Thomas W. Phillips

Laboratory: Stored-Product Insects Res. Unit

Address: USDA, ARS Dept. of Entomology
University of Wisconsin
Madison, WI 53706

CRIS #: 3655-43000-002-00D

Telephone No.: 608-262-0814

FAX #: 608-262-3322

A. Research Accomplishments (up to 5) in Last Five Years:

1. Determined that grain volatiles synergistically enhance activity of pheromone in *Tribolium castaneum* and *Sitophilus oryzae*.

2. Demonstrated attraction and oviposition by female *Plodia interpunctella* in response to secretions from conspecific larvae and cracked grain.

3. Identified the defensive secretions of predatory pirate bugs, *Xylocoris flavipes* and *Lyctocoris campestris*. Evaluated terpene alcohols for fumigant toxicity.

4. Evaluated chemical food protectants against the pest mite *Tyrophagus putrescentiae*.

B. Research Objectives for Next Five Years:

Overall significance is to developed methods to manage stored-product insects that will reduce or eliminate the use of conventional insecticides while maintaining high levels of productivity and quality.

A. Isolate and identify attractants and oviposition stimulants for female *P. interpunctella*.

B.Isolate, identify, and formulate volatiles from grain that are attractants or pheromone synergists for stored-product insects.

C. Develop food-safe methods incorporating natural products to control mite infestation in processed foods.

D. Develop insect-resistant food packaging that utilizes natural repellents, deterrents and toxicants.

C. Current and Future Cooperators (ARS and Others):

ARS: David Weaver - volatile plant compounds as insecticides
Others: Michael Strand (U. Wisc.) - chemical ecology of *Plodia*; Richard ffrench-Constant (U. Wisc.)- insecticide resistance and molecular genetics; Francis Webster (SUNY) - chemical isolation,identification & synthesis of natural products.

D. Potential Uses of Research Findings:

Work on attractants, pheromones, and natural pesticides should have direct applications as tools for pest management. The food industry is in great need of alternatives to concentrated pesticides and in new monitoring tools.

E. Technology Transfer and End-Use Strategies and Opportunities:

Currently working with pest management and food companies to develop certain technologies that will benefit the food industry. One CRADA is in place to develop control tactics for mites in pet food. A second CRADA is planned to develop insect-resistant, natural product-based, food packaging.

F. Thoughts on Research Needs:

Stored-products are facing a potential pest management crisis. Few residual insecticides are legally available for use and insects are evolving resistance to those in place. Fumigants are also being lost to regulation. Effective alternatives to these compounds need to be found. Together with improved sanitation, natural pesticides should be explored to a greater extent.

G. Chemical methods Employed:

Insect tissues or plant materials are extracted in organic

solvents, or volatiles are collected by headspace aeration and entrainment onto solid-phase adsorbents. Fractionation and purification is by LC and micro-preparative GC. Analysis is by GC/FID and GC/MS, and via collaboration with chemists.

H. Bioassay Methods Employed:

Insect attractants are assayed via flight in a wind tunnel and via pedestrian orientation in multiple choice pitfall arenas (static air) or upwind movement in Y-tube designs. Repellents are assayed by simple petri dish tests in which aversion to treated surfaces is scored. Feeding deterrents are assayed by incorporation into food and comparing feeding on treated and untreated foods. Toxicants are tested by contact/fumigant assays in petri dishes or feed-through studies by incorporation into diet.

Name: Gary Puterka

Laboratory: Appalachian Fruit Research Station

Address: USDA, ARS,
Kearneysville, WV 25430

CRIS: 1931-22000-004-00D

Telephone: 304-728-2361

FAX: 304-728-2340

A. Research Accomplishments in Last 5 Years:

1. Pilot test of sugar ester extracted from wild tobacco *Nicotana sp.* against pear psylla.

B. Research Objectives for Next 5 Years:

1. Purpose: Control of pear psylla with sugar exters extracted from wild tobacco, *Nicotana sp.*, and its effect on non-target insects.

2. Significance: Pear psylla has some degree of resistance to all labeled insecticide used against it. These sugar esters offer a new alternative insecticide that appears to be specific to homopteran, thus leaving natural enemies in tact.

3. Constraints: This product may be difficult to commercialize because it is processed from plants, not synthesized.

C. Current and Future Cooperators:

John Neal - Beltsville, USDA
Ray Severson and R. Chortyk - Athens, USDA
Tom Unruh - Yateina, WA
Helmat Reidle - Univ. Oregon, Hood River

D. Potential Users of Research Findings:

Evaluate the efficency of this compound for pear psylla control and lead to compounds registration and labeling for pear psylla control.

E. Technology Transfer and End-Use Strategies:

This area is being done by Georga Buta, USDA, Beltsville, MD.

F. Thoughts on Research Needs:

More vigorous field testing against various homopteran pests and effects on natural enemies and beneficial insects is needed.

G. Chemical Methods Employed:

H. Bioassay Methods Employed: Pear psylla

Name: Walter E. Riedell

Laboratory: Northern Grain Insects Research Laboratory
RR #3
Brookings, SD 57006

CRIS #: 5447-22000-006-00D

Telephone #: 605-693-5207

Fax #: 605-693-5240

A. Research Accomplishments in Last Five Years:

1. Evaluate the toxicity of naturally-occurring and synthetic loline alkaloid derivatives for insecticide activity against aphids, fall armyworm, and European corn borer. Work done in collaboration with chemists from the National Center for Agricultural Utilization Research.

2. Evaluate the activity of naturally occurring and experimental plant growth regulators in reversing Russian wheat aphid-induced leaf rolling. Work done in collaboration with private industry.

3. Investigate the insecticidal activity (against corn rootworm larvae) of isobutylamides isolated from purple coneflower (*Echinacea purpurea*) flower heads. Work done in cooperation with scientists at South Dakota State University.

B. Research Objective for Next Five Years

To identify and determine metabolic pathway of chemicals produced by corn roots that deter or stimulate corn rootworm larval feeding activity. Identify processes in the corn plant that can be altered to deter corn rootworm larval feeding.

1. Purpose: To produce corn germplasm that resists feeding activity of corn rootworm larvae, an insect pest that costs corn producers roughly $1 billion in grain yield loss and pesticide treatment costs each year.

2. Significance: Successful completion of the research outlined above provides new knowledge of the chemical

ecology of corn rootworm/plant relationships. Such knowledge could be transferred to plant breeders and molecular biologists to construct plants that resist corn rootworm larval feeding.

3. Constraints: Need the talents of a chemist/biochemist to aid in isolating and identifying substances that have the desired activity. Need the talents of plant breeders and molecular biologists to transfer substances to (or remove substances from) corn roots.

C. Current and Future Cooperators:

Close cooperation with a behavioral entomologist here at the NGRIL is just beginning. Potential future cooperators are located at South Dakota State University (located 1 mile from NGIRL) and at the National Center for Agricultural Utilization Research

D. Potential Uses:

Use of corn rootworm resistant plants by corn producers would reduce grain yield loss to corn rootworm insects without using insecticides. This will reduce production costs and potential pesticide environmental contamination.

E. Technology Transfer and End-Use Strategies and Opportunities:

Research is just beginning, so technology transfer is currently impossible at this time.

F. Research Needs:

Need the talents of a chemist/biochemist to aid in isolating and identifying substances that have the desired activity. Need the talents of plant breeders and molecular biologists to transfer substances (or remove substances) from corn roots.

G. Chemical Methods Employed:

TLC, HPLC, GC, and GC-MS are all available for use by the project.

H. Bioassay Methods Employed:

Surface and soil olfactometers to study larval feeding behavior have been established. Techniques for observing larval feeding behavior *in vitro* (dissection microscope) and *in vivo* (soil mini-rhizotron) can be adapted with the purchase of new equipment.

*

Name: David C. Robacker

Laboratory: Subtropical Agricultural Research

Address: 2301 S. International Blvd.
Weslaco, TX 78596

CRIS #: 6204-43000-005-00D

Telephone No.: 210/565-2647

FAX #: 210/565-6652

A. Research Accomplishments (up to 5) in Last Five Years:

1. A chemically defined protein-type attractant for both males and females was developed.

2. A chemically defined attractant for both males and females was developed from host fruit volatiles.

3. Attractiveness of pheromone to both males and females in the field was demonstrated.

4. It was shown that bacteria of numerous species from at least five families are generally attractive to Mexican fruit fly.

5. Differential attractiveness of host-fruit and protein-type baits was linked to adult nutrition and physiology.

B. Research Objectives for Next Five Years (Brief Description):

The objectives are to develop an attractant for a dry trap that is superior to currently used baits and to understand how it works.

1. Purpose: The purpose of the work is to replace the standard McPhail trap containing protein bait currently used to monitor populations of *Anastrepha* fruit flies with a new, more effective dry trap with a chemically defined bait that can emit attractive amounts of chemicals for at least twice as long as currently used traps.

2. Significance: Such a dry trap should be less expensive than the McPhail trap both in actual cost of traps and in manpower needed to run trapping programs. More importantly, more effective traps translate into earlier detection of fly populations and therefore faster and less expensive eradication.

3. Constraints: Current slow-release technology is inadequate. I also need more analytical chemistry support for identification of bacteria-produced attractants and cooperators for field testing of new traps with the various *Anastrepha* species.

C. Current and Future Cooperators (ARS and Others):

ARS: R. Flath (Albany, CA); R. Heath (Gainesville, FL); E. Jang (Hilo, HI)

APHIS: A. Martinez, K. Esau (Mission, TX); D. Chambers (Guatemala City, GUA)

Industry: W. Denton (AgriSense); J. Jenkins (Scentry)

D. Potential Uses of Research Findings:

Discussed above was replacement of the expensive McPhail trap/protein bait trapping system with less expensive dry traps containing long-lasting chemically defined attractants. In addition, this research will increase our understanding of complex relationships of environmental and physiological factors affecting attraction of fruit flies to various baits. Such knowledge could enable users to make informed choices regarding which baits to use under a specific set of conditions and could aid researchers working with other species of Tephritidae to better understand their systems. Finally, this research may introduce new chemical classes as attractants that may function in novel ways.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Development of slow-release technology for these types of chemicals will be conducted by industry during the next year. The attractant technology will be transferred to my industry partner for commercial production by 1995. Large

scale pilot testing perhaps in California with CDFA or in Texas with TDA is anticipated for 1996-97.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

N/A

G. Chemical Methods Employed:

Methods used in my laboratory include various volatile collection techniques, on-column and Grob injection capillary GC, and HPLC.

H. Bioassay Methods Employed:

A rapid laboratory bioassay called a "cage-top" bioassay is used for initial screening of most chemicals. A flight chamber type wind tunnel and local field testing with sterile, released Mexican fruit flies are used to evaluate chemicals and mixtures that seem promising. Field testing in Mexico and Guatemala is employed to evaluate attractants deemed best in local field testing.

Name: Ray F. Severson

Laboratory: Phytochemical Research Unit

Address: USDA/ARS/RRC
P. O. 5677
Athens GA 30613

CRIS #: 6612-21410-005-00D

Telephone #: 706-546-3495

Fax #: 706-546-3454

A. Research Accomplishments (up to 5) in Last Five Years:

1. Different types of sugar ester (sucrose[SE] and glucose[GE])were isolated from *Nicotiana* species, characterized, and found to be toxic to the Sweetpotato whitefly(SPWF) and aphids. Methodology was developed to extract and isolate large quantities of sugar esters from *N.* species for field testing as biorationals against the SPWF and aphids.

2. Hydroxyacyl-nornicotines were isolated from *N.*species, characterized,and found to be toxic to SPWF, aphids, and tobacco hornworms.

3. Diterpenes were isolated from *N.* species, characterized, and several were found to be toxic to aphids, and/or inhibited plant fungal diseases.

4. Fall armyworm and corn earworm larval antibiosis factors in centipede grass were isolated and identified.

5. Ovipositional stimulants were isolated and identified for tobaco budworm from *N. Tabacum* and other *N.* Species and for Sweetpotato Weevil from sweetpotato leaves.

B. Research Objectives for Next Five Years (brief description; also fill in attached table using brief descriptors):

Host plant resistance factors in *N.* species, corn, and other plants.

1. Purpose. Identify compounds in plants which naturally control insect and disease damage.

2. Significance: The development of biorationals for the control of agricultural pests and resistant germplasm for crop production.

3. Constraints: ARS patent processes are too slow. Need funding for ARS cooperator M. G. Stephenson at Tifton GA to provide needed plant material and to investigate agronomic conditions to commercially produce plants for the isolation of biorational materials. Need more bioassays. Need Post Doc. to assist in characterization of active compounds.

Biorationals isolated from *N.* species.

1. Purpose: To develop methodology for the on site extraction of cuticular components from *N.* species which would assist in the commercialization of the biorational products for the control of the SPWF, aphid and plant fungal diseases and other value added products.

2. Significance: Safe natural products are needed for the control of plant pests. An alternate crop for production in the Southeast could be developed.

3. Constraints: ARS patent process is too slow. Need funding for cooperating Univ. of GA agricultural engineer at Tifton and Athens GA to assist in the development of extraction equipment.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Entomologists: ARS-D. H. Akey, R. Coleman, K. D. Elsy, D. M. Jackson, J. W. Neal, Jr., G. J. Puterka, A. Simmons, M. T. Smith, D. A. Wolfenbarger and B. Wiseman; University- A. W. Johnson (Clemson), G. A. Herzog and R. M. McPherson (UGA), L. S. Osborn and P. A. Stansly (U of FL), D. G. Riley (Texas A&M) and N. C. Toscano (U of CA).

Chemists: ARS-O. T. Chortyk and G. Pomonis.

Plant Physiologists: ARS-H. G. Cutler; University-S.Kays (UGA) and G. Wagner(U of KY)

Geneticists: ARS-G.Pittarelli and V.A. Sisson; University-M.Neilsen (U of KY).

D. Potential Uses of Research Findings:

The identified biorationals have potential for commercial use as agricultural chemicals. The host plant resistant factors can be incorporated into commercial germplasm.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Currently have Confidentiality Agreements with several Ag chemical companies interested in *N.species* biorationals for SPWF and aphid control.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Funds are needed to support the testing of *N.* components for insecticidal and fungicidal activities This would include the growing of large quantities of plant materials for formulations, and field bioassays.

G. Chemical Methods Employed:

Methods of isolation and identification are developed for the determination of each specific plant. Generally, extracts of plant materials are purified by solvent partition, column chromatography, chromatotron chromatography and LH-20 or reverse phase liquid chromatography. Identifications are based on UV, HPLC, GC/MS, and NMR data.

H. Bioassay Methods Employed:

Bioassays are conducted by cooperating USDA and University entomologists on specific target insects, including tobacco aphids, tobacco budworms, tobacco hornworms, pear psyllas, sweetpotato whiteflies, etc., using isolated fractions or compounds.

Name: Jeffrey P. Shapiro

Laboratory: U.S. Horticultural Research Laboratory

Address: 2120 Camden Road
Orlando, FL 32803

CRIS #s: 6617-24000-006-00D; 6617-22000-007-00D

Telephone No.: (407) 897-7376

FAX #: (407) 897-7309

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovered whitefly-induced proteins in squash silverleaf-afflicted cucurbits.

2. Characterized citrus root pyranocoumarin contents and responses to weevil feeding damage.

3. Discovered and characterized naringenin (flavonone) hydroxylase activity in citrus callus and callus microsomes.

4. Isolated/characterized coumarin- and riboflavin-binding hemolymph storage proteins from the citrus root weevil, *Diaprepes abbreviatus*.

B. Research Objectives for Next Five Years (brief description):

Objectives: Through bioassay and chemistry, identify natural plant products (flavonoids, coumarins, and other small compounds; and proteins) with potential to confer resistance to citrus and vegetable crops against target pest insects; identify, characterize, and isolate enzymatic systems responsible for synthesis of natural products that confer resistance; begin transgenic incorporation into citrus and vegetable crops.

1. Purpose: To both discover and utilize natural products as sources of plant resistance to insects.

2. Significance: The first practical transgenic systems for crop resistance are now being developed. However, only a few natural product candidates have been identified for

future use. Many more are needed, with efficacy against pests of a broad range of host plants.

3. Constraints: Few technically trained personnel, and inability to retain them for more than 1-2 years on temporary positions without benefits.

C. Current and Future Cooperators (ARS and Others):

ARS: R.T. Mayer, W.J. Schroeder, P.D. Greany, P. Shaw, R. Niedz.

D. Potential Uses of Research Findings:

Identification and isolation of genes coding for key enzymes in natural product synthesis are initial steps toward protection of plants with intrinsic natural products. Discovery of useful natural products and their genes will be essential in replacing synthetic insecticides with engineered resistance in plants.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Technology transfer will entail handoff of technologies (e.g. useful genes) to other ARS personnel (e.g. for genetic incorporation into citrus at USHRL). Alternatively, industrial firms may license patented discoveries. Time frame is long term; useful products may be discovered within 1 year, but implementation will require at least five years.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

Besides much-needed longterm technical and professional staffing, a very important need not addressed by ARS is efficient transfer of scientific information. For example, direct electronic access to NAL holdings, the RMIS and CRIS databases, and to databases of scientific literature (e.g. Chemical Abstracts) would greatly enhance research efficiency. More importantly, direct access to a more detailed database of ARS scientific personnel and their expertise would enhance potential teamwork.

G. Chemical Methods Employed:

Spectroscopy (UV-vis, FTIR, GS-MS, fluorescence), chromatography (HPLC, TLC and radial TLC, GC, GC-MS), electrophoresis (PAGE, 2-D PAGE, CE, Western blotting)

H. Bioassay Methods Employed:

Diaprepes abbreviatus: Insect forced-feeding assay, semi-defined diet assay, protein binding assays; *Bemisia tabaci*: whole plant feeding assay, adult membrane feeding assay; *Anastrepha suspensa*: adult feeding assay, larval semidefined diet assay.

Name: Ted N. Shaver, Research Chemist

Laboratory: Crop Insect Pests Management Research Unit

Address: USDA, ARS, SPA, SCRL
Crop Insect Pests Management Research Unit
Rt. 5, Box 808
College Station, TX 77845

CRIS #: 62202-22000-004-00D

Telephone #: 409-260-9351

Fax #: 409-260-9386

A. Research Accomplishments in Last Five Years:

1. Determined that chickpea terminals and fruiting forms emit an oviposition attractant for *Heliothis virescens* and that the attractant is extractable with methylene chloride.

2. Prepared extracts and volatile collections for ten species of plants that have been demonstrated as attractive to *Helicoverpa zea* and other noctuids by cooperators. Eleven compounds have been identified that are common to orange and grapefruit blossoms. An additional five compounds were identified from orange that were not present in grapefruit. Twenty-two other compounds have been tentatively identified from orange blossoms, eight of which were also contained in grapefruit. Several mixtures of chemicals identified from hexane extracts of *Gaura drummondii*, *Gaura suffulta*, or *Gaura longiflora* have some activity to *H. zea* in laboratory bioassay test. Also, some of the headspace volatile collections of *Gaura* species have stimulated directed flight and proboscis extension in a wind tunnel using *H. zea* as test insects.

B. Research Objectives for Next Five Years:

Objective: Identify attractants for insect control

1. Purpose: Identify volatile chemicals from plants attractive to *Helicoverpa zea* and other noctuids and determine activity of individual chemicals and combinations of chemicals in laboratory and field bioassays.

2. Significance: Chemicals that act as feeding attractants to *H. zea* and other noctuids can be incorporated into an attracticide together with feeding stimulants and toxicants to control source populations of adult insect pests, resulting in reduced pesticide used and selective placement of insecticide to reduce contamination of edible plant parts.

3. Constraints:

Objective: Attractants for monitoring insect population

1. Purpose: Chemicals that are identified as feeding attractants for *H. zea* and other noctuids will be tested for efficacy in trapping wild populations of insects to monitor field populations.

2. Significance: Currently, insect detection and monitoring systems utilizing traps baited with pheromone captures only one sex, usually the male, of the insect of interest. Preliminary studies have demonstrated that plants with adult feeding attractant activity and extracts of these plants capture both sexes in traps.

3. Constraints:

C. Current and Future Cooperators:

ARS: P. D. Lingren, J. D. Lopez, K. R. Beerwinkle (College Station, TX); J. R. Raulston (Weslaco, TX); B. A. Leonhardt (BARC).

D. Potential Uses of Research Findings:

Successful completion of research on plant feeding attractants will result in development of technology for controlling field populations of insects by targeting adults instead of larval stages. This should result in less use of pesticides and selective placement of insecticides so as to not contaminant edible portions of the plant.

E. Technology Transfer and End-Use Strategies and Opportunities:

Technology transfer will be accomplished through publication of technical manuscripts and use of CRADA's and other

strategies as progress warrants.

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

Volatiles will be identified as possible attractants for *Helicoverpa zea* from extracts and headspace volatile collections from attractive plants. Identification of chemicals will be made by GC and GC-MS coupled with IR and NMR when applicable. Chemicals not available through commercial channels will be synthesized for testing.

H. Bioassay Methods Employed:

Bioassays will be conducted in cooperation with K. R. Beerwinkle and are covered in his report.

Name: Philip E. Shaw

Laboratory: Citrus & Subtropical Products Lab.

Address: 600 Avenue S, Northwest (P.O. Box 1909)
Winter Haven, FL 33881

CRIS #: 6621-41430-001-00D

Telephone #: 813/293-4133

FAX #: 813/299-8678

A. Research Accomplishments in Last Five Years:

1. Work on effects of gibberellic acid (GA) on peel oil content in grapefruit has shown that treatment with GA significantly increases the yield of peel oil at maturity.

2. Use of GA spray on grapefruit has retarded normal decrease in limonin content in peel with increasing maturation (in some cases). Limonin is a known antifeedant for some insects, but its effect on the Caribbean Fruit Fly (Caribfly) is unknown.

B. Research Objectives for Next Five Years:

The objective is to supplement the entomological finding that use of GA delays attack by the Caribfly in maturing grapefruit by trying to determine which peel components contribute to peel resistance to attack.

1. Verify for a second year the statistical increase in peel oil content in grapefruit after GA treatment.

2. Continue montoring limonin content in control vs GA-treated peel to accurately determine level of limonin present when peel becomes susceptible to attack by the Caribfly.

3. Study composition of lemon peel to help determine reason for high resistance to Caribfly in this citrus cultivar.

C. Current and Future Cooperators:

ARS: P. Greany (Gainsville, FL); R. McDonald, R. Schroeder,

J. Shapiro, R. Mayer (Orlando, FL).

Foreign: A. Malavasi (Sâo Paulo, Brazil); M. Aluja (Veracruz, Mexico).

D. Potential Uses of Research Findings:

The correlation of either limonin content or peel oil content with degree of resistance to attack by the Caribfly could lead to an analytical procedure for monitoring fruit for resistance to Caribfly attack prior to shipment out of state. Use of GA to increase peel oil content in citrus has great economic potential for commercial recovery of peel oil from grapefruit, lemon, and lime, where oil sales are a multimillion dollar industry yearly in the U.S.

E. Technology Transfer and End-Use Strategies and Opportunities:

Mainly, this work supports the impending approval of GA as an approved treatment of grapefruit in Florida to prolong the current fly-free zones by six to eight weeks. Any use specifically to increase the peel oil content in citrus fruit would be a three to four year committment requiring additional SY's and larger scale processing of fruit for more accurate yields of oil recovery.

F. Thoughts on Research Needs:

Potential antifeedant activity of limonin involving the Caribfly or other fruit flies should be explored. Limonin is available for testing, but the manpower needed for screening for biological activity is lacking.

G. Chemical Methods Employed:

Use of HPLC, GC, GC/MS

H. Bioassay Methods Employed:

None

Name: Michael T. Smith

Laboratory: Southern Insect Management Lab
Jamie Whitten Research Laboratory

Address: P.O. Box 345
Stoneville, MS 38776

CRIS #: 6402-22000-019-00D

Telephone: 601-686-5289

FAX: 601-686-5421

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identification of key biological parameters (insect biology and behavior), and development of bioassay methodology for evaluation of host plant resistance to the pecan aphid species complex (blackmarginned aphid, *Monellia caryella*; yellow pecan aphid, *Monelliopsis pecanis*; black pecan aphid, *Melanocallis caryaefoliae*).

2. Initial evaluation of potential sources of host plant resistance to the three aphid pest species on pecan has been conducted among the Juglandaceae family of nut trees [hickory (*Carya*) and walnut (*Juglans*) species] native to North America, as well as among a select group of 7-10 pecan cultivars. Both biological (developmental rate, reproductive rate, etc.) and behavioral (feeding behavior) studies have been performed and are still in progress. These studies are being conducted at various intervals over the growing season (various phenological stages of plant development). To date, a number of Juglandaceae species have been identified as resistant to the three pecan aphid species.

3. Development of methodology for the extraction and analysis of leaf chemistries potentially associated with host plant resistance to pecan aphids. Extraction and analysis have been performed (GC/MS, HPLC) among the Juglandaceae species. Extraction and analysis of leaf chemistries among the pecan cultivars is in progress.

4. Analysis of the volatile and cuticular chemistries of the

pecan fruit (GC/MS, HPLC), as a function of phenological development, has provided candidate compounds for evaluation as host attractants and/or ovipositional stimulants for the primary nut pests in pecan (pecan weevil (*Curculio caryae*), hickory shuckworm (*Cydia caryana*), pecan nut casebearer (*Acrobasis nuxvorella*).

5. Re-examination of the hickory shuckworm sex pheromone (GC/MS) has provided some evidence, based upon EAD responsiveness, of one, possibly two, additional compounds in the pheromone.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop a biologically based management system for the key pest species of pecan based upon a fundamental understanding of the factors governing host plant selection by each pest species. This broad objective results from the acute awareness of the interrelatedness of management decisions directed towards each pest species, as well as a need to develop biologically based, sustainable, and environmentally friendly management systems for crop production.

1. Objective:
Systematic identification of host plant resistance to the three pecan aphid species among the North American hickory and walnut species (Juglandaceae) and pecan cultivars.

2. Analysis of the surface and interior chemistries among the Juglandaceae species and pecan cultivars investigated in objective 1.1 (GC/MS and HPLC).

3. Evaluation of plant chemistries (identified in objective 1.2) for biological activity as antifeedants or toxicants for control of pecan aphids (via artificial diet assays)

Significance:

1. These investigations should: (1) identify a diversity of plant species/cultivars which are resistant to pecan aphids, (2) identify a diversity of natural products of plant origin that are responsible for the host plant resistance, and (3) identify the resulting type of resistance that these natural products mediate, and

therefore their potential utility.

Constraints:

1. Geographic (Juglandaceae and pecan cultivar orchards are located in Byron, GA) and financial constraints.

2. Development of bioassay techniques for evaluation of surface and interior leaf chemistries.

C. Cooperators:

B.W. Wood, C.C. Reilly and W.L. Tedders, S.E. Fruit and Nut Research Lab, USDA-ARS, Byron, GA; R.F. Severson, R.J. Horvat, and J.A. Robertson, Phytochemical Research Unit, USDA-ARS, Athens, GA.

Potential Uses of Research Findings:
Research findings will: (1) identify sources of host plant resistance to the pecan aphid species among the Juglandaceae species and existing pecan cultivars, which will aid in breeding programs; (2) lead to the development of new pecan management strategies where multiple-cultivar orchards would be established by designed in order to reduce aphid damage, as well as reduce the rate at which aphids develop the ability to counter the natural defenses of the plants; and (3) identify the specific natural products associated with host plant resistance, provide an understanding of the biological activity of the natural products, and lead to the development of the appropriate application technology.

2. Objective:
As an integral part of our investigations of the sex or aggregation pheromone of the pecan weevil, *Curculio caryae* (a serious nut pest of pecan), identification and evaluation of weevil and plant chemistries for biological activity as natural products for attraction, monitoring, and/or mating or ovipositional disruption of the pecan weevil.

Significance:
These investigations will identify the specific plant chemistries which mediate host finding, feeding or ovipositional stimulation of the pecan weevil. Similar

studies have led to the discovery of monitoring and/or attracticide technologies.

Constraints:
Development of bioassay techniques for evaluation of chemistries which mediate host and mate finding (aggregation), and oviposition.

Cooperators:
G. Greis and H. Pierce, Simon Fraser University, Burnaby, Canada; R.J. Horvat and R.F. Severson, Phytochemical Research Unit, USDA-ARS, Athens, GA.

Potential Uses:
Research findings will identify the specific natural products associated with host plant and mate finding, as well as those natural products serving as ovipositional stimulants. Potential uses include the discovery of natural products that will enable growers to monitor, deter, and/or directly control the pecan weevil without the use of conventional synthetic insecticides.

3. Objective:
In concert with re-evaluation of the sex pheromone of the hickory shuckworm, *Cydia caryana* (a serious nut pest of pecan), the ultimate purpose of the research is to identify and evaluate plant chemistries for biological activity as natural products for attraction, monitoring, and/or mating or ovipositional disruption of the hickory shuckworm.

Significance:
These investigations will identify the specific plant chemistries which mediate host finding, and ovipositional stimulation of the hickory shuckworm. Similar studies have led to the discovery of monitoring and/or mating disruption technologies.

Constraints:
Development of bioassay techniques (flight tunnel, electroantennogram, and field) for evaluation of chemistries which mediate host finding and oviposition; Financial constraints for periodic travel to locations where large populations exist.

Cooperators:

G. Greis and H. Pierce, Simon Fraser University, Burnaby, Canada; R.J. Horvat and R.F. Severson, Phytochemical Research Unit, USDA-ARS, Athens, GA.

D. Potential Uses:

Research findings will identify the specific natural products associated with host plant and mate finding, as well as those natural products serving as ovipositional stimulants. Research findings will also provide a detailed understanding of the biological activity of the natural product, which is essential for development of the appropriate application technology without the use of conventional synthetic insecticides.

E. Future/Additional Objectives:

1. Development of application technology for the utilization of the identified natural products.

2. Technology transfer and end-use strategies.

Name: Maurice E. Snook

Laboratory: Phytochemical Research Unit

Address: USDA-ARS, Russell Research Center
P.O. Box 5677
Athens, GA 30613

CRIS #: 6612-21410-005-00D

Telephone No.: 706-546-3597

Fax #: 706-546-3454

A. Research Accomplishments

1. Developed HPLC method for the natural pesticide, maysin, the *Helicoverpa zea* (corn earworm) antibiosis factor in corn silks.

2. Identified new corn germplasm sources with high silk maysin that can be used in breeding studies.

3. Identified several new natural flavonoids in corn that are active against the corn earworm.

4. Identified several natural polyphenols in tobacco roots that inhibit the growth of *Phytophthora parasitica* var. *nicotiana* (black shank fungus) and determined important structural/activity relationships of their analogues.

5. Isolated, identified, and quantitated the flavonols of the *Nicotiana* species and Plant Introductions that should aid breeders in lowering polyphenols.

B. Objectives:

To isolate, identify, and quantify biologically active compounds from *N. tabacum* (tobacco) and other *Nicotiana* species, *Zea mays* (corn), peanuts, and other plants or crops that are responsible for insect and disease resistance.

1. Purpose:

Investigate which plant chemicals are responsible for the

observed bioactivity and determine if they possess agronomically useful properties such as biorationals, insecticides, bactericides, fungicides, nematocides, plant growth regulators and other value added products.

2. Significance:

Development of environmentally safe bio-rationals with potentially other useful properties is a major goal of ARS. Development of insect and disease resistant crops that will lower cost and increase yields.

3. Constraints:

None.

C. Current and Future Cooperators:

1. Dr. Verne A. Sisson, Research Geneticist, Crops Research Laboratory, USDA-ARS, Oxford, NC.
2. Dr. Alexander S. Csinos, Plant Pathologist, Head, Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA.
3. Dr. Neil W. Widstrom, Research Geneticist, Insect Biology and Population Management Research Laboratory, Tifton, GA.
4. Dr. Billy R. Wiseman, Research Entomologist, Insect Biology and Population Management Research Laboratory, Tifton, GA.
5. Dr. Robert Lynch, Research Entomologist, Insect Biology and Population Management Research Laboratory, Tifton, GA.
6. Dr. Murray Blum, Professor, Department of Entomology, University of Georgia, Athens, GA.
7. Dr. Albert Culbreath, Plant Pathologist, Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA.
8. Dr. Stanley S. Kays, Research Horticulturist, Dept. of Horticulture, University of Georgia, Athens, GA.
9. Dr. Patrick F Byrne, Research Geneticist, Dept. of Agronomy, University of Missouri, Columbia, MO.
10. Dr. Lana Reid, Research Geneticist, Agriculture Canada, Plant Research Center, Ottawa, Canada.

D. Potential Uses of Research Findings.

Industry will use the findings of this research to produce plant based products. Farmers will use developed pest resistant germplasm to produce food and fiber, will grow specialty crops which produce discovered natural products and along with other consumers, will use developed natural product pesticides. Entomologists will used identified plant semiochemicals in the development of insect management systems. Information and technology will be used by other natural product scientists.

E. Technology Transfer and End-Use Strategies and Opportunities:

Release of insect and disease resistant germplasm is a major goal. Release of corn earworm resistant germplasm is hoped for within 2-years.

F. Thoughts on Research Needs:

Access to bioassay evaluation of isolated compounds for a wide range of potential uses. The number of test systems available is limited. Now, individual scientist need to find others willing to test their compounds. A central listing of persons, willing to perform bioassay tests, should be available to any scientist who would like to have his/her compounds tested.

G. Chemical Methods Employed:

Plant material is either frozen immediately after sampling or slurried in methanol to stop enzymatic degradation of active components. Usual procedure for isolation of active components is evaporation of methanol and extraction of aqueous phase with n-butanol is utilized to isolate flavonoids. Components are than isolated by a combination of silicic acid and preparative reverse-phase chromatography. Identification of components is by IR, MS (El and FAB), UV, hydrolysis, synthesis.

H. Bioassay Methods Employed:

Corn earworm (*Helicoverpa zea*) and the fall armyworm *Spodoptera frugiperda* laboratory bioassays performed on isolated natural products or model compounds by Dr. B.R. Wiseman, Tifton, GA. Sap beetle species (*Carpophilus lugubris* and *Carpophilus freemani*) tested by Dr. Patrick F. Dowd, USDA-ARS, Peoria, IL.

Name: Roy Teranishi

Laboratory: Western Regional Research Center

Address: 800 Buchanan Street
Albany, CA 94710

CRIS #: 5325-41000-009-00D

Telephone No.: 510-559-5659

FAX #: 510-559-5828

A. Research Accomplishments (up to 5) in Last Five Years:

1. Verified that ammonia, in hydrolyzed vegetable protein, is the major attractant of walnut husk flies (*Rhagoletis completa*), very effective attractant predominantly for females.

2. Angelica seed oil has been shown to be a very effective attractant for male walnut husk flies.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop natural product attractants for tephritids, general and species and sex specific. Natural product compounds will be tested with walnut husk flies because they are endemic to this region. Activities will be checked with other tephritid species at other locations.

1. Purpose: The ultimate purpose of the research is to find better lures for tephritids for survey and control, especially of Medflies. It will be very useful to have general and species specific lures as well as lures which attract predominantly males or females.

2. Significance: Natural product lures and control compounds are biodegradable and will have less damaging effects on the environment as do synthetics.

3. Constraints: None.

C. Current and Future Cooperators (ARS and Others):

FS: S. B. Opp (California State University-Hayward), S. Gothilf (Agric. Res. Org., Bet Dagan, Israel), J. Hendrichs (IAEA, Seibersdorf, Austria).

D. Potential Uses of Research Findings:

Patents
Use by APHIS in surveying and controlling insects.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

From the time of discovery and complete identification of useful natural product, it will take a year or two of field studies. Technology transfer will be rapid, probably in three or four years after discovery, because we will be working with APHIS.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

From my experiences with various groups, there is a very great need of cooperation between disciplines. Entomologists seem to think that chemists are technicians who should simply find or make compounds for their convenience. Chemists seem to think entomologists are technicians to report back biological activities. There must be genuine respect for each others capabilities and knowledge, with frequent exchange of information, or there will be little progress.

G. Chemical Methods Employed:

The most modern isolation, separation, and identification methods must be utilized, with close cooperation with entomologists to follow up on active materials isolated.

H. Bioassay Methods Employed:

Even with established conventional trapping systems used, there must be close cooperation between the entomologist and chemist to adjust dosages and ways to administer the lures in order to determine and optimize degree of attractancy.

Name: J. David Warthen, Jr.

Laboratory: Insect Chemical Ecology Laboratory

Address: Bldg. 007 Rm. 312, BARC-West
10300 Baltimore Avenue
Beltsville, MD 20705-2350

CRIS#: 1275-27000-086-00D

Telephone: 301-504-6981

FAX: 301-504-6580

A. Research Accomplishments in Last 5 Years:

1. Isolated and identified male medfly attractive components in *Litchi chinensis* stems and *Ficus spp*. stem exudates and developed the laboratory bioassay to detect these components.

2. Isolated and identified furanosesquiterpenoids of *Commiphora myrrh* oil and evaluated extracts of *C. erythraea* as larvicides and repellents for ticks.

3. Determined deterrence coding by a larval Manduca chemosensory neurone mediating rejection of a non-host plant, *Canna generalis*.

4. Investigated the effects of neem-seed extract on Colorado potato beetle and *Eyprepocnemis plorans*; investigated the effects of azadirachtin on *E. plorans* and immune-depression in *Rhodnius prolixus*.

B. Research Objectives for Next 5 Years:

Objective 1: To isolate and identify toxic components in leafy spurge.

1. Purpose: To find components that are toxic to the western corn rootworm.

2. Significance: Control of the western corn rootworm.

3. Constraints: None

Objective 2: To isolate and identify attractants from

dallisgrass and willow.

1. Purpose: To find natural attractants for *Helicoverpa*.

2. Significance: Control of *Helicoverpa*.

3. Constraints: None

Objective 3: To isolate and identify attractants from butterfly bush.

1. Purpose: To find new attractants for Hawaiian fruit flies.

2. Significance: Monitoring and controlling Hawaiian fruit flies.

3. Constraints: None

Objective 4: To isolate a medfly ovipositional stimulant degradation product from a natural sesquiterpene.

1. Purpose: To find an ovipositional stimulant for the medfly.

2. Significance: A medfly ovipositional stimulant would be a definite asset in combination with a toxic agent for a control program.

3. Constraints: None

Objective 5: To isolate an attractant from red coffee berries.

1. Purpose: To find a natural attractant for medfly.

2. Significance: Monitoring and controlling the medfly.

C. Cooperators:

Neal Spencer, John Davis, Bill Lusby (Objective 1) - source of leafy spurge, bioassay, and mass spectral data, respecively.

Ted shaver and Barbara Leonhardt (Objective 2) - wind tunnel bioassay and GC/mass spectral analyses, respectively.

Eric Jang (Objective 3,4, & 5) - bioassay

D. Potential Uses of Research Findings:

For monitoring and controlling insects: western corn rootworm, *Helicoverpa* and Hawaiian fruit flies.

E. Technology Transfer and End-Use Strategies:

Transfers to Industry will occur where appropriate.

F. Research Needs:

Quicker turnaround on bioassays.

G. Chemical Methods Used:

H. Bioassays Methods Used:

Name: David K. Weaver

Laboratory: Stored-Product Insects R. & D.

Address: 3401 Edwin St.
Savannah, GA 31405

CRIS#: 401-6605-050(17)

Telephone No.: 912-651-3512

FAX #: 912-651-3500

A. Research Accomplishments (up to 5) in Last Five Years:

1. Demonstrated that linalool, a common component of floral scents, is a moderately toxic contact and fumigant insecticide for certain stored product pests.

2. Milling of dried leaves of certain aromatic plants released volatile toxicants that controlled selected stored product pests, with the milled leaves being directly added as a carrier of toxicant(s).

3. Demonstrated that the essential oil of *Tetradenia riparia*, a mint used in traditional African medicine, is an effective seed dressing to protect dried beans from bruchid attack.

4. Evaluated the insecticidal potential of root, foliar and floral essential oil extracts (SSDE) of Mexican marigolds against an economically important bruchid.

   * All were completed prior to current ARS appointment. Work conducted at Montana State as a postdoctoral research associate (Dec. 89 - Jan. 92).

B. Research Objectives for Next Five Years (brief description):

Current CRIS appointment is primarily ecological in orientation, however, an evaluation of the attractant and repellant potential of stored corn/fungal communities will be undertaken.

1. Purpose: This information will enhance modelling of stored product insect populations by:

(a) providing compounds that can be used to monitor population growth.
(b) indicator compounds that signal imminent insect dispersion may be identified. These may also serve as management or control tools.

2. Significance: Speculative at this point and addressed in the above category.

3. Constraints: This is only a minor component of our modelling project, so available personnel are limiting.

C. Current and Future Cooperators (ARS and Others):

ARS: T. Phillips (Madison, WI), R. Bartelt (Peoria, IL), L. Zettler, (Savannah, GA), J. Baker (Savannah, GA).

University: L. Jackson (Montana State, Bozeman, MT), W. Bertsch (Univ. of Alabama, Tuscaloosa, AL)

Industry: D. Hynson (Nurture Biotech)

D. Potential Uses of Research Findings:

Earlier research on volatile plant components as insecticides may provide new structure/activity leads. These may show particular promise as leads for novel fumigants.

Historically, little emphasis has been placed on behaviorally active compounds that protect crops or commodities without killing the pest. This type of control technology for stored products can be developed while investigating the chemical ecology of stored grain insect/fungal communities. Ultimately, repellant compounds or novel attractants for trapping may be developed.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

U.S. Patent Application Serial No. 07/801,817. Insecticidal or insect behaviorally active preparations from aromatic plants. Patent allowed Nov., 1993.

* co-inventor, prior to ARS employment.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

ARS should develop a comprehensive team plan for research on natural product based control that encompasses residue analysis and non-target organism testing immediately after biological activity has been characterized. This structured approach will better serve our mission statement. The current focus appears to be patenting of novel structures with demonstrated activity. Does this really benefit agriculture? Is there a clear mechanism for efficiently getting information to get to the point of technology transfer?

G. Chemical Methods Employed

Organic liquid chromatography
Gas chromatography/mass spectroscopy
Steam distillation; Simultaneous steam distillation and extraction
Solvent extraction

H. Bioassay Methods Employed

Behavioral and toxicological insect bioassays *in vitro* and on commodity.

Names: Donald T. Wicklow & Patrick F. Dowd

Laboratory: National Center for Agricultural Utilization Research

Address: 1815 N. University Street
Peoria, IL 61604

CRIS #: 3620-42000-009-06T

Telephone No.: 309-681-6243

FAX #: 309-681-6686

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identified fungal sclerotia as a source of novel insecticides and other biologically active fungal metabolites.

2. Discovered over fifty (50) natural products which possess previously unreported chemical structures including eight (8)new ring systems.

3. Seven (7) U.S. Patents issued.

B. Research Objectives for Next Five Years (brief description):

We are conducting general studies of the chemistry of fungal sclerotia as sources of new antiinsectan natural products that protect these survival structures from soil-inhabiting fungus-feeding arthropods.

1. Purpose: There is an urgent need for new sources of insecticides, and leads to insecticides (i.e. new chemotypes) because many insects are developing resistance to existing products and environmentally tolerable replacements to these pesticides are becoming fewer in number.

2. Significance: Several novel compounds have been discovered with oral activity against *Helicoverpa zea* comparable to that of the commercial synthetic insecticides malathion (an organodithiophosphate) and permethrin (a pyrethroid).

3. Constraints: None presently. This project has sufficient funding through 1995, with support from the National Science Foundation and the Biotechnology Research and Development Corporation (BRDC),Peoria, IL.

C. Current and Future Cooperators (ARS and Others):

J.B. Gloer, Department of Chemistry, University of Iowa, Iowa City,IA. (Co-Principal Investigator).

Individual 'member companies' of BRDC.

BRDC outlicencing to non-member companies, with permission of the 'member companies.'

D. Potential Uses of Research Findings:

The novel compounds we have discovered through our insect bioassay guided studies may have other relevant biological activities (e.g. pharmaceutical). One of the best known examples is the ergot fungus *Claviceps purpurea* which produces a sclerotium that has been exploited for pharmaceutically important ergot alkaloids (e.g. ergotamine). The pharmaceutical industry apparently has not expanded beyond the ergot fungus to screen the sclerotia of other fungi for their biologically active metabolites.

E. Technology Transfer and End-Use Strategies and Opportunities:

Technology transfer is on-going with company tests of our new compounds. We can not predict the outcome of these tests and are unable to suggest a timeframe for the eventual "end-use" of these compounds.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Chemical ecologists, lateral thinkers able to cross organism-group boundaries, are critical to the formation of successful interdisciplinary natural products discovery teams. Novel ideas, with the prospect of finding interesting new chemical structures with relevant biological activities, will always outperform mindless screening programs in attracting outstanding natural products chemical support. Cooperation with outstanding natural product chemists at

Universities offers greater flexibility to the ARS scientist and brings additional funding options. Concentration of resources at a single institute, or within a single 'national program,' may afford greater visability but could drain resources from numerous successful programs funded through the competitive grants process. The BRDC model allows relevant direct feedback from industry while efficiently performing technology transfer, freeing the scientist to follow new research leads.

G. Chemical Methods Employed:

Department of Chemistry, University of Iowa - HPLC systems, countercurrent chromatography system, gas chromatograph, Bruker 600-MHz FT-NMR (AMX-600), VG ZAB-HF high-resolution mass spectrometer, IBM 9098 GC/FT-IR, Perkin-Elmer 2400 CHN analyzer, etc.

H. Bioassay Methods Employed:

Diet incorporation; topical; leaf discs, mode of action.

Parasite and Pathogen Control

* Indicates that the information is also relevant to other research areas and has been included as such.

*

Name: Stephen Beckstrom-Sternberr
James A. Duke

Laboratory: National Germplasma Resource Laboratory

Address: USDA, ARS, B-003 R-227, BARC-West
Beltsville, MD 20705

CRIS: 275-21000-057-00D

Telephone: 301-504-5419

FAX: 301-504-5536

A. Research Accomplishments in Last 5 Years:

Natural Pesticide Database Developed

B. Research Objectives for Next 5 Years:

1. Purpose: To discover or popularize natural pesticide as alternative crop to protectants.
2. Significance: Natural pesticides and medicinal compounds are becoming more popular with consumer and USDA should be more consumer-driven.
3. Constraints: Political, Security. (in foreign locations)

C. Current and Future Cooperators and their Contributions:

Weed Science Laboratory (USDA) (Growing Greenhouse Crops)
CRC Press (published database)

D. Potential Uses of Research Findings:

Crop diversification in the humid tropics.
Reduction in narcotic exports from Peru

E. Technology Transfer and End-Use Strategies:

Trying, with civilian conterparts, also to develop marketable alternative crops, including pesticides, medicine, hypoallergenic rubber, low fat chocolate, antioxidant salad dressings.

F. Thoughts on Research Needs:

Critical evolution would favor synergism between pesticidal compound, in plants. Yes, USDA and Industry go for the

silver bullet.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Name: John M. Bland

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-018-00D

Telephone No.: (504) 286-4279

FAX #: (504) 286-4367

A. Research Accomplishments in Last Five Years:

1. First Synthesis of the natural fungicide, iturin.
2. New synthesis of B-amino acids/synthesis of iturinic acid.
3. Synthesis of iturin analogs for SAR studies.
4. Synthesis of tentoxin analogs for SAR studies.
5. New synthesis of the natural herbicide, tentoxin.

B. Research Objectives for Next Five years:

1. Purpose: To determine the structural and spatial components of: A) Iturin and B) Tentoxin, needed for biological activity. Produce an economically feasible A) fungicide, B) herbicide with the environmentally-safe properties of the parent compound.
2. Significance: Development into a viable replacement for the commercially available fungicides being removed from the market because of their toxic residues on foods and contamination of ground water.
3. Constraints: Manpower

D. Potential Uses of Research Findings:

New commercial products:
A) Fungicide B) Herbicide

G. Chemical Methods Employed:

Solution and solid phase peptide synthesis methods.

H. Bioassay Methods Employed:

A) Fungal inhibition zone on agar plate.
B) Lettuce seedling chlorosis.

Name: Karen D. Burkhead

Laboratory: National Center for Agricultural Utilization Research

Address: USDA ARS
1815 N. University St.
Peoria, IL 61604

Cris #: 3620-41000-031

Telephone No: 309-681 -6287

FAX #: 309-681-6686

A. Research Accomplishments (up to 5) in Last Five Years:

1. (This CRIS, 1992-93) Isolated and identified a putative germination inhibitor of wheat from *Pseudomonas fluorescens* 2-79.
2. (This CRIS, 1992-93) Isolated and identified the antifungal compound pyrrolnitrin from *Pseudomonas cepacia* B37w and demonstrated its antifungal activity against causal agents of potato dry rot.
3. (This CRIS, 1992-93) Detected from one to eight antifungal compounds from each of twenty bacterial strains by bioautography.
4. (Previous CRIS, 3620-41000-029-02 T, 1990-92) Screened 670 microbial cultures for aryl hydroxylase activities of industrial importance. Identified 47 *Aspergillus* strains and several cultures from other genera able to regiospecifically hydroxylate several model aromatic compounds of industrial interest.
5. (University of Iowa, College of Pharmacy, 1989-90) Isolated and identified aurofusarin from *Fusarium graminearum* and prepared chemical derivatives of this poorly soluble pigment. Reported for the first time C-13 nmr data and biological activity of these naphthoquinone dimers against Gram (+) and Gram (-) bacteria.

B. Research Objectives for Next Five Years (brief description): to isolate and identify antibiotics

1. Purpose: The purpose is to isolate and identify bacterial antifungal compounds which have been detected in potential biocontrol bacteria, using fermentation, extraction, chromatography, and spectrocopic techniques.
2. Significance: a. Knowledge of antibiotic production is important for forrnulation of the cultures as biological control products. b. The antifungal compounds themselves may have useful veterinary and human applications, as well as many crop protection possibillities.
3. Constraints: a. One year remains in my current post-doctoral appointment. b. The overall project goal is development of the bacteria as biocontrol agents. The best biocontrol agents are not the best antibiotic producers.

C. Current and Future Cooperators (ARS and Others):

ARS: D. Schisler and P. Slininger (NCAUR, Peoria, IL).

D. Potential Uses of Research Findings:

Microbial cultures may be used for biocontrol of potato dry rot fungi and other crop pathogens.

Antifungal compounds have potential applications in crop protection, veterinary uses, and treatment of human systemic and/or topical infections.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes

Many agrochemical and pharmaceutical companies demonstrated interest in this project at the 206th ACS National Meeting in Chicago. Aside from the development of cultures as biocontrol agents, there may be separate uses for discovered antifungal compounds. If novel structures are identified within the next year, technology transfer arrangements for new antifungal leads may be readily developed, since the market for antifungal compounds is rapidly growing.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Within ARS, natural product leads might be screened for additional activities. For example, antifungal compounds that protect potatoes from dry rot may be useful against other fungal pathogens of other crops, and may have insecticidal activities as well. For information transfer and technology transfer within ARS, a directory of natural products research activities/leads would be useful. I would be willing to work on the creation of such a directory.

G. Chemical Methods Employed:

Extraction from microbial fermentation, chromatography (flash column, TLC, HPLC), spectroscopy (MS, NMR). May use GCMS, UV, IR.

H. Bioassay Methods Employed:

Whole potato, bioautography assays for antifungal compounds.

Name: Michele R. Carter
Laboratory: Floral and Nursery Plant Research Unit
Address: Rm 208 B-004 BARC-West
10300 Baltimore Avenue
Beltsville MD 20705-2350

CRIS #: 1230-22000-003-00D
Telephone No.: 301-504-6413
FAX#: 301-504-5096

A. Research Accomplishments in the last 5 years:

1. Demonstration of the efficacy of various botanical extracts as potential foliar fungicides. The activity of botanical seed oils were shown to be effective in whole plant bioassay systems using bean, carnation, and snapdragon rusts and hydrangea powdery mildew. These oils delayed lesion development and sporulation of the blackspot fungus on rose using an *in vitro* detached leaf bioassay.

2. Demonstration of the efficacy of various organic amendments to control or suppress fungal damping-off pathogens. Some solid natural product amendments added to soilless growing medium have shown significant reduction in the number of plants affected by damping-off fungal pathogens.

B. Research objectives for next 5 years(brief description):

1. Purpose: To evaluate the activity of botanical extracts against foliar fungal pathogens. More specifically to evaluate the extracts potential use in integrated pest management systems and determine their mechanism of action.

2. Significance: Progress in the area of screening botanicals for biological activity has increased the probability of developing products for use in pest management programs and the potential for patents and registrations.

3. Constraints: The major constraints to the future of this project are financial compounded with the lack of technical and collaborational assistance.

C. Current and Future Collaborators:

Current:
Private sector: W. R. Grace and Co. is supporting the evaluation of new botanicals for biological activity.

University and Extension: Collaboration with the University of Maryland for evaluation of botanicals in field research

plots.

Future:
Collaboration with Dr. Charles Wilson, USDA, Kernysville, W VA to develop a database of literature on botanicals with antifungal activity.

D. Potential uses of research findings:

Botanical extracts may be utilized in integrated pest management schemes by: 1) replacing harmful agrochemicals or those lost by failure to reregister, 2) mixing botanical products with a reduced rate of currently labelled fungicides, or 3) alternating fungicide and botanical extract spray applications.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

CRADA's (1 yr.)
Patent (protection/licensing) (2-5 yr.)
Commercialization/marketing/sourcing (5-10 yr.)

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

G. Chemical Methods Employed:

Performed by W.R. Grace and Co..

H. Bioassay Methods Employed:

Conducted greenhouse whole plant bioassays, field trials, and *in vitro* bioassay systems.

NAME: O. T. Chortyk

LABORATORY: Phytochemical Research Unit

ADDRESS: USDA, ARS, R. B. Russell Agricultural Research Center
P. O. Box 5677, Athens, GA 30613

CRIS #: 6612-21430-001-00D

TELEPHONE #: 706-546-3424

FAX #: 706-546-3454

A. Research Accomplishments (up to 5) in Last Five Years:

Syntheses and characterization of sugar esters and determinations of their biological activities.

B. Research Objectives for Next Five Years (brief description: also fill in attached table using brief descriptors):

Purpose:

(a) To identify biologically active components in plants and to develop synthetic method for their large scale production.
(b) To produce new biologically active compounds.

2. Significance: To develop environmentally safe biorationals.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

C. Current and Future Cooperators and their Contributions (ARS and others):

ARS: R. F. Severson, D. M. Jackson, G. Pittarelli, M. E. Snook, G. Puterka, R. Horvat, D. Barnard, H. Cutler, M. Stephenson

Other: A. Johnson, Clemson
N. Toscano, U. Cal.

D. Potential Users of Research Findings:

Developed biosafe pesticides against sweet potato whiteflies, psylla, aphids, other insects will be most welcomed by farmers and environmentalists.

E. Technology Transfer and End-Use Strategies and Opportunities (include predictive timeframes):

1993--Started cooperative research with industry.

1994--Possible CRADAS with one or more companies

1995-97--large-scale production of sugar esters, field tests, possible commercial product.

F. Thoughts on Research Needs:

None

G. Chemical Methods Employed:

Standard methods of analysis (GC, GC/MS, HPLC) are coupled with purification methods involving column chromatography, chromatotron separations and LH-20 column chromatography.

H. Bioassay Methods Employed:

Insect bioassays on developed compounds are/will be conducted by cooperators, employing insects such as whiteflies and aphids.

Name: William J. Connick, Jr. and Donald J. Daigle

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-22000-001-00D

Telephone No.: (504) 286-4527

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Established germination rate as a criteria for the successful formulation of *Alternaria cassiae*, a mycoherbicide for the weed, sicklepod (*Cassia obtusifolia L.*).
2. Developed improved invert emulsion formulations for mycoherbicides, reducing dew requirements.
3. Developed a new formulation concept using wheat flour to make granules called "PESTA" containing biocontrol fungi and nematodes.

B. Research Objectives for Next Five years (brief description):

To discover and develop new or improved formulations for biocontrol agents.

1. Purpose: Some primary goals are to control the weed, hemp sesbania, in fields, to improve formulation technology with the use of a mycoherbicide with a cheap, practical delivery system, and to develop an effective granular formulation containing entomopathogenic nematodes. Methods to increase shelf life of the biocontrol agents are being investigated.

2. Significance: Lack of suitable formulations is a major constraint to further acceptance of biopesticides. Biocontrol products can reduce the amount of pesticide applied to crops such as soybeans and thus reduce the farmers cost and/or reduce the effect of pesticides on the environment. Biopesticide products with adequate shelf life will help the farmer and homeowner.

3. Constraints:

Products with adequate shelf life and efficacy are scarce. Only three mycoherbicide products have been commercialized in the last 15 years.

Use of insect-killing nematodes is a promising application,

but improved formulations are needed. Granular products would fill an important niche. More formulation research by industry, universities, and ARS is needed.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Numerous ARS scientist engaged in biocontrol research. Among them are Dr. Doug Boyette, Stoneville, MS, USDA, ARS; and Dr. Mark Jackson, Peoria, IL, USDA, ARS. Dr. P.C. Quimby, Jr., Bozeman, MT and Dr. Bill Nickle, BARC-W.

D. Potential Uses of Research Findings:

To broaden the base of cooperative research and to bring biocontrol formulations closer to commercial acceptance.

E. Technology Transfer and end Use Strategies and Opportunities:

Two companies have applied for exclusive rights to Pesta containing insect-killing nematodes. With suitable effort, a biocontrol product could be marketed within 3 years.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Strengthen the research on formulation and application technology.

G. Chemical Methodologies in Use:

Numerous chemical pesticide formulations are available, but the technology used does not usually apply to delicate, living biocontrol agents.

H. Bioassays in Use:

Bioassays are usually performed by the biological scientists we cooperate with. However, we sometimes evaluate weed control on seedlings in the greenhouse.

Name: Horace G. Cutler

Laboratory: Microbial Products Research Unit

Address: Richard B. Russell Research Center
P.O. Box 5677
Athens, GA 30613

CRIS #: 6612-41000-001-00D

Telephone No.: (706) 546-3378

FAX No.: (706) 546-3250

A. Research Accomplishments in Last Five Years:

1. Isolation and identification of a natural product fungicide. The material is presently in field trials to control *Armillaria* in Kiwifurit and *Pinus radiata* and to control silverleaf in Asian pears and ornamentals. It is biodegradable and safe.

2. Isolation of a bioremediating organism that aerobically breaks down PCPs (under patent).

3. Isolation and identification of (-) harzianopyridone, a fungal natural product with herbicidal properties.

4. Isolation of botcinolide, a novel natural product herbicide.

5. Antimicrobial and plant growth regulating properties of sucrose esters.

B. Research Objectives for Next Five years:

The objective is to isolate, characterize, and utilize biodegradable natural products from microorganisms for agricultural and other uses.

1. Purpose: To isolate, identify and use as herbicides, antimicrobials/antivirals, natural products from microorganisms for practical use as biodegradable chemicals (environmentally benign agents) and/or pharmaceuticals.

2. Significance: To protect the enviroment, to produce quality pesticide free food and to produce value-added products from fermentation.

3. Constraints: Technical - Need NMR facilities and easy access to X-ray crystallography. Need FAB-MS and HRP-MS.

C. Current & Future Cooperators:

- Dr. Robert A. Hill, HortResearch, New Zealand. Microorganisms and field tests. $25,000
- Dr. L. Cheah, Food Research Ltd, New Zealand. Post harvest applications of natural products.
- Dr. H. Rhothitha, HortResearch, New Zealand. Entomology
- Dr. Karst Hoogsteen, Merck Therapeutic Research. X-ray crystallography.
- Dr. Gary Newton, Univ. of Georgia, X-ray crystallography
- Dr. Stephen Cutler, College of Pharmacy, Mercer Univ. Chemical Synthesis
- Dr. John Jacyno, College of Pharmacy, Ohio Northern Univ. Toxicology.

D. Potential Uses of Research Findings:

Biodegradable, environmentally safe agricultural chemicals for uses both pre and post harvest to protect crops and products. These will protect consumer health and will protect the export market. In addition, we expect financial profit from these developments.

E. Technology Transfer and End-Use Strategies and Opportunities:

To industry, for development, either before or after the patent process. Some of our materials are already undergoing field trials (Timeframe: now and < 10 years).

F. Thoughts on Research Needs:

There are several natural product sources that are not being tapped because of lack of financial support and, especially, a lack of imagination in spending and obtaining funds.

G. Chemical Methods Employed:

Column chromatography, Prep HPLC, HPLC, TLC, Spinning Plate, UV, FT, R, 1HNMR, ^{13}CNMR, LRP_{MS}, FABMS, X-ray crystallography.

H. Bioassay Methods Employed:

Etiolated wheat coleoptile bioassay; phytotoxicity tests on greenhouse-grown plants; antibacterial and antifungal bioassays; antitumor bioassays (extra-mural).

Name: Paul Engel

Laboratory: Southern Regional Research Center

Address: 1100 Robert E. Lee Blvd.
New Orleans, LA 70124

CRIS #: 6435 41000 018 00D

Telephone #: (504) 286-4375

FAX #: (504)286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Nikkomycin, a chitin synthase inhibitor, is a safe but potent fungicide. Inactivated (tagged) nikkomycin biosynthetic genes of Streptomyces tendae with transposon Tn4560 to facilitate isolation of these genes. Isolated three transposon-tagged nikkomycin non-producing mutants.

2. Cloned DNA that flanks the insertion site of Tn4560 from one of the nikkomycin non-producing, transposon-tagged mutants.

3. Did gene disruption to verify that cloned DNA flanking insertion site of Tn4560 specifies nikkomycin biosynthesis.

B. Research Objectives for Next Five Years (brief description):

Clone (isolate) entire cluster of genes required for nikkomycin biosynthesis.

1. Purpose: To introduce nikkomycin biosynthetic genes into microorganisms (biocontrol agents) that are antagonistic to fungal diseases to make them more effective agents. To manipulate nikkomycin biosynthetic genes so this substance can be produced economically by fermentation. To introduce nikkomycin genes into plants (transgenic plants) so they can protect themselves against fungal disease and post harvest losses.

2. Significance: Nikkomycin is a safe but potent fungicide that could be effective in preventing pre/post-harvest losses due to fungi.

3. Constraints:

(a) attitude among ARS management that this approach is too "blue sky," i.e., impractical.

(b) questions about product safety if product derived by genetic engineering.

(c) questions about product safety if product has been treated with an "antibiotic."

C. Current and Future Cooperators (ARS and Others):

David Kingston, Dept. of Chemistry, Virginia Tech (Blacksburg, VA) is currently doing MS and NMR to determine structure of substances accumulated by mutants.

D. Potential Uses of Research Findings:

Safe and effective fungicide to increase shelf-life of feed/food and effective selfprotectant against fungal diseases in transgenic plants.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Personnel from Crop Genetics International, Miles Laboratories, and Native Plants, Inc., have expressed interest.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

Need increased enthusiasm and confidence among managers that biotechnology is practical.

G. Chemical Methods Emp1Oyes

1. HPLC used in house; needed to go outside for MS and NMR.

2. DNA required in my research is generally not available in commercial kits. I have to harvest DNA needed for experiments myself.

H. Bioassay Methods Employed

Routinely use *Mucor hiemalis* to bioassay for fungicidal activity.

Name: Susan L. Fricke Meyer

Laboratoory: Nematology Laboratory

Address: USDA/ARS/Nematology Lab
Bldg. 011A Rm. 153, BARC-West
10300 Baltimore Avenue
Beltsville, MD 20705-2350

CRIS: 1275-13240-001-00D (To be replaced with 1275-13220-003-00D

Telephone: 301-504-5091

FAX: 301-504-5589

A. Research Accomplishments in Last Five Years:

ARS coworkers (Huettel, Jaffe, DeMilo, Hayes, and Rebois) isolated and identified a sex pheromone from soybean cyst nematode. To date, this is the only sex phermone identified from a plant-parasitic nematode. Pheromone analogs were synthesized. Meyer and Huettel tested selected compounds in the greenhouse for ability to act as nematode management agents. Field tests were conducted in collaboration with Crop Genetics International. In addition, the pheromone and one analog have been tested in the greenhouse and field in combination with a fungus for biocontrol of soybean cyst nematode. Patent applications have been filed on this technology, a Cooperative Research and Development Agreement has been signed with industry, and the company has a license to the technology.

B. Research Objectives for Next Five Years:

The broad objective is to obtain knowledge about the activity of the fungus and the bioregulatory compounds, and to determine affects of these agents on plant-parasitic nematodes.

1. Purpose: To gain information about the fungus, the pheromone, and the pheromone analogs, and the ways these agents affect nematode populations. This might eventurally lead to development of a management agent for soybean cyst nematode.

2. Significance: Soybean cyst nematode is resopnsible for more than $250 million in soybean production losses per year in the United States. Vanillic acid is the first sex pheromone to be isolated and identified from a plant-parasitic nematode, and this compound or related compounds may be effective for decreasing nematode populations.

3. Constraints: ARS personnel assigned to the project consist of 1 SY and 1 technician, and occasional student help. More

personnel are needed for time-consuming greenhouse and field tests. In addition, greenhouse space is limited. The number of greenhouse tests that can be run each year is far fewer than needed to answer important questions.

C. Current and Future Cooperators (ARS and Others):

ARS: David Chitwood (BARC), Baruch Shasha (Peoria, IL).

APHIS: Robin Huettel (MD).

Industry: Crop Genetics International

D. Potential Uses of Research Findings:

The research will provide basic information about activity of fungi and bioregulatory compounds that affect the nematode life cycle. Possible uses include development of management agents for plant-parasitic nematodes.

E. Technology Transfer and End-Use Strategies and Opportunities:

A CRADA has been established with industry. The CRADA expires in 1994. A license agreement has been signed with industry.

F. Research Needs:

ARS has best personnel available to conduct research on biocontrol of plant-parasitic nematodes. In addition, greenhouse space is limited. Consequently, work progresses slowly and many important aspects of the project are not being addressed at this time. For example, a number of the compounds have never been tested for effects on nematodes. In addition, studies are needed on mode of action of the fungus and the bioregulatory compounds, rhizosphere competence of the agents, and longevity in the soil.

G. Chemical Methods Employed:

Currently, no one working on the project is a chemist.

H. Bioassay Methods Employed:

Nematodes and potential management agents are applied near plant roots in greenhouse soil. Nematode populations are counted to determine efficecy of treatment.

Name: Howard Harrison and Joseph Peterson

Laboratory: USDA-ARS, U. S. Vegetable Laboratory

Address: 2875 Savannah Highway, Charleston, SC 29414

CRIS #: 6659-22000-005 and 6659 22000 006

Telephone: (803) 556-0840

Fax: (803) 763-7013

A. Research Accomplishments in Last Five Years

1. Demonstrated that sweetpotato interference with yellow nutsedge and other weeds is due in part to allelopathy.

2. Isolated a sweetpotato root periderm compound that is highly inhibitory to yellow nutsedge root growth and velvetleaf and proso millet seed germination. Selectivity between species in seed germination inhibition was also observed.

3. Field and greenhouse studies indicated that the growth of allelopathic sweetpotato clones were reduced less by weed interference than non-allelopathic clones.

4. Demonstrated yellow squash components are highly inhibitory to proso millet seed germination and may contribute to its allelopathic properties.

B. Research Objectives for Next Five Years

1. Describe traits (allelopathic and morphological) that give sweetpotatoes greater competitiveness against weeds.
2. Isolate for identification by cooperating chemists other allelochemicals from sweetpotato.
3. Isolate for identification by cooperating chemists allelochemicals from squash.
4. Develop laboratory techniques to identify pest resistant and allelopathic crop genotypes.

C. Current and Future Cooperators.

ARS; P. Dukes (Pathologist), J. Bohac (Geneticist), J. Thies (Nematologist)

USVL, B. Horvat (Chemist), J.K. Porter (Chemist) Athens, W. Lusby (Chemist) Beltsville.

University; M. Sheppard (Entomologist), Clemson., D. LaBonte (geneticist),

C. Motsenbacher (Weed Scientist), LSU.

D. Potential Uses of Research Findings:

This research is part of a multidisciplinary, collaborative effort with the broad objective of investigating the biochemical bases for host plant resistance in vegetable crop species and isolating and identifying compounds present in vegetable crops that are responsible for allelopathy and disease and insect resistances. Potential benefits of this research are; (1) discovery of new natural products that may be useful for pest control, (2) development of "in vitro" methods to identify resistant or allelopathic crop genotypes, (3) development of bioassays for identifying pesticidal natural products or resistant plant genotypes, (4) provide information useful in identifying "resistance genes" that can be transferred to other plants by conventional or biotechnological methods.

E. Technology Transfer and End Use Strategies and Opportunities:

Several agrichemical companies have expressed interest in investigating sweetpotato defense compounds as natural products for pest control; however, no formal agreements have been made. The most likely end use of this research is identification of constituents in vegetables that contribute to pest resistances. This will allow plant breeders to quickly quantify pest resistance levels, a process which now takes several years. The first strategy is long term and would probably take at least 10 years to reach the market. The second strategy could be implemented as soon as methods to quantify resistance factors are developed.

F. Thoughts on Research Needs:

There is a wealth of information known to subsistence farmers in undeveloped areas about the use of living or dead plants to suppress weeds and other pests. A concerted effort to study these practices and to identify and preserve the species used could provide leads toward the identification of pesticidal natural products and species useful as cover crops for pest suppression in an IPM program.

G. Chemical Methods Employed.

HPLC, GC, column chromatography, TLC.

H. Bioassay Methods Employed

Various seed germination tests, yellow and purple nutsedge root growth, *Fusarium oxysporum* mycelial growth, root knot nematode egg hatching and survival, diamondback moth and Fall armyworm growth and survival.

NAME: R. J. Horvat

LABORATORY: Phytochemical Research Unit

ADDRESS: USDA, ARS, R. B. Russell Research Center
P. O. Box 5677
Athens, GA 30613

CRIS #: 6612-21410-005-00D

TELEPHONE #: 706-546-3194

FAX #: 706-546-3454

A. Research Accomplishments (up to 5) in Last Five Years:

1. Volatile constituents from the flowers of 5 *Nicotiana* species have been identified by GC/MS.

2. Volatile compounds of squash leaves and squash trichromes have been identified by GC/MS. These compounds are being currently evaluated for oviposition and attractant stimulants to the female moth (*Diaphania nitidalia*).

3. Blueberry and deerberry volatiles have been identified by GC/MS and will be evaluated for attraction of the adult fly (*Rhagoletis mendax*).

B. Research Objectives for Next Five Years:

The objective is to isolate and chemically identify new biologically active (volatile and semivolatile) compounds from plant parts and flowers of *Nicotiana* species and specific tobacco varieties, with the objective of characterizing environmentally acceptable agrochemicals that may find use as insect attractnats or repellants, insect predator attractants, antimicrobials and natural pesticides.

1. Purpose: The ultimate purpose of this research is to gain knowledge of the compounds responsible for insect (beneficial and pests) attraction to specific plants.

2. Significance: This information could provide plant breeders with the knowledge required to breed plants with minimum levels of these insects pest attractants. Also, plants could be produced to attract beneficial insects.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):
None

C. Current and Future Cooperators and their Contributions (ARS and others):

J. K. Peterson and H. Harrison, U. S. Vegetable Laboratory, USDA/ARS, Charleston, SC.

S. D. Pair, South Central Ag. Research Laboratory, Lane, OK

J. A. Payne, Fruit and Tree Nut Research Laboratory, Byron, GA

D. M. Jackson, Crops Laboratory, Oxford, NC

W. S. Schlotzhauer, O. T. Chortyk, H. Cutler, S. Nottingham, USDA/ARS, R. B. Russell Agricultural Research Center, Athens, GA

D. Potential Users of Research Findings:

Plant breeders, entomologists and commercial companies producing biocontrols.

E. Technology Transfer and End-Use Strategies and Opportunities (include predictive timeframes):

This work has not progressed to the point where Technology Transfer, etc. are applicable.

F. Thoughts on Research Needs (not been addressed in other Agencies or at State Levels:

None

G. Chemical Methods Employed:

Leaf volatiles were obtained by hexane and methylene chloride dipping of leaves. These extracts were concentrated and subjected to vacuum steam distillation/hexane extraction in order to remove high molecular weight plant waxes. Hexane concentrates were analyzed by GC/MS and GC for identification of components.

H. Bioassay Methods Employed:

Wind tunnel experiments are currently being set up for evaluation of individual compounds as insect attractants. Colonies of *Cardiochiles Nigriceps* and *Heliothis virescens* have been established for use in wind tunnel experiments.

Name: Sidney E. Kunz
J. A. Miller

Laboratory: Knipling-Bushland U.S. Livestock Insects Research Laboratory, ARS, USDA

Address: 2700 Fredericksburg Road
Kerrville, Texas 78028

CRIS #: 6205-32000-007-00D, 009-00D, 010-00D, 011-00D

Telephone #: 210-792-0304, 0321, 0303

FAX #: 210-792-0314

A. Research Accomplishments (up to 5) in Last Five Years:

1. Determined efficacy and optimal dosage for ivermectin, moxidectin and doramectin for flies and ticks affecting livestock.

2. Developed delivery systems for oral and SC injection for ivermectin.

3. Developed vaccine for cattle grub.

4. Demonstrated efficacy of neem for horn flies.

5. Determined efficacy and optimal dosage for IGR's against flies.

B. Research Objectives for Next Five Years:

1. Purpose: Explore and develop natural products for control of livestock pests; develop vaccine for cattle grub and mange mite; determine efficacy and means of application of 2nd generation avermectins; screen and develop microbiols for fly control; develop delivery systems for these agents.

2. Significance: Reduce dependence on synthetic pesticides; increase safety of compounds used; decrease chances of environmental contaiminants; broaden the arsenal of available control tools.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

   Availability of effective materials.
   FDA regulations in registration of new products.
   Commercialization of minor use product for livestock pest control.

C. Current and Future Cooperators and their Contributions:

Private industry - make available new products.
ARS laboratories - as new products are developed.

D. Potential Uses of Research Findings:

Addition of new replacement materials for livestock pest control.
New products to manage pesticide resistnace.

E. Technology transfer and End-Use Strategies and Opportunities:

License of patented technology to private industry for marketing.
Establishment of CRADAs with private industry.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Safety of products developed

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Name: Alan R. Lax

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-018-OOD

Telephone No.: (504) 286-4382

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Tentoxin inhibits chloroplast envelope ATPases.
2. Polyphenol oxidase has transit sequence making it similar in that respect to other known chloroplast proteins synthesized in the cytoplasm.
3. *Alternaria alternata* genomic DNA has homology to other known cyclic peptide synthetases.

B. Research Objectives for Next Five Years:

(Brief title of each objective)

1. Purpose:

Identify and clone gene(s) for tentoxin biosynthesis.

Increase production of tentoxin to commercially feasible levels.

2. Significance:

Economically feasible production of biorational pesticides.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

Technical
Regulatory (if using engineered organisms directly). Patent

C. Current and Future CooDerators and their Contributions (ARS and Others):

1. None presently
2. If genes are cloned possible collaboration with any of several agrichemical companies.

D. Potential Uses of Research Findings:

1. Produce tentoxin in economically significant quantities.

2. Develop synthetic compounds to affect chloroplast transport with specificity of tentoxin.
3. Transform organisms/host plants for delivery of herbicide.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Opportunities for technology transfer include fermentation industry and seed companies. Tentoxin end-use potential includes fermentation for increased production of the product per se while further potential exists to incorporate tentoxin biosynthetic capabilities into crop plants allowing them to synthesize herbicides directly. Technology to produce tentoxin through fermentation of engineered organisms should be developed within the next two to three years, while incorporation of the biosynthetic genes into plants is a relatively long term goal.

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

UV, IF, NMR, MS, Chromatography.

H. Bioassay Methods Employed:

Molecular biology, enzymes, plants.

Name: Dr. James C. Locke

Laboratory: Floral and Nursery Plants Research Unit

Address: Rm. 208, B-004, BARC-West
10300 Baltimore Avenue
Beltsville, Maryland 20705-2350

CRIS #: 1230-22000-003-00D

Telephone #: 301-504-6413

FAX #: 301-504-5096

A. Research Accomplishments (up to 5) in Last Five Years:

1. Demonstration of the efficacy of neem seed compositions as foliar fungicides.

2. Patents issued for process and use of clarified neem seed oil.

3. Submission of registration package to EPA for clarified neem seed oil.

4. Demonstration of efficacy of various botanical extracts as potential foliar fungicides.

B. Research Objectives for Next Five Years (brief description; also fillin attached table using brief descriptors:

1. Purpose: To evaluate botanical products as potential replacements/ alternatives for synthetic pesticides.

2. Significance: The reduction of synthetic pesticide utilization in pest management programs.

3. Constraints (e.g.) regulatory, patent, fiscal, technical, disciplinary, commercialization etc.):

The major perceived constraints to the project are fiscal and the need for a multi-disciplinary cooperative network for evaluation/development.

C. Current and Future Cooperators (ARS and Others):

Current: Private Sector (W.R.Grace) - fiscal, patent, process development, registration.
University/Extension - plot evaluations.
National Park Service - plot evaluations.

Future: Private Sector (same as Current)

Chemists - a.i. determination, formulation, structural analysis. Entomologists/Nematologists - concurrent evaluations.

D. Potential Uses of Research Findings:

Identification of active botanical products is the first step in developing safe pesticidal products. Formulation, application technology, and field efficacy testing must follow. The involvement of private sector partners, who will determine economic feasibility, must be fostered early in the process.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

CRADA's (1 year)
Patent (protection/licensing) (2-5 years)
Commercialization/marketing/sourcing (5-10 years)

F. Thoughts on Research Needs (not being addressed in other Agencies
or at State levels):

Coordination of disciplines - botanist, chemist, microbiologist, entomologist, nematologist, pathologist, physiologist/weed scientist, focused on objective of identification/development/commercialization of N.P.

Requires central mandate and support of NPS.

G. Chemical Methods Employed:

N/A

H. Bioassay Methods Employed:

Whole plant-disease bioassays are employed. Selection of pathogens is determined by activity of botanical product.

Name: Edward C. Lulai

Laboratory: Potato Research Laboratorv

Address: 311 5th Ave NE
P.O. Box 113
East Grand Forks, MN 56721

CRIS #: 3650-43000-006-OOD

Telephone No.: 218-773-2473

FAX: 218-773-2207

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identified lipoxygenase (LOX)-null tuber cell cultures.
2. Employed LOX-null cultures to demonstrate LOX system requirement in hypersensitive response.
3. Developed histochemical probes specific for detection of each major suberin component during wound healing.
4. Related deposition of each suberin component to resistance to penetration by bacterial soft rot and fungal dry rot.
5. Patent applied for on natural control of potato sprouting and improvement of raw product market quality.

B. Research Objectives for Next Five years (brief description):

The broad objective is to solve priority potato quality problems affecting the viability and competitiveness of U.S. industries in domestic and developing export markets; this includes use of endogenous natural products of potato which may be used to further control damage related disease problems impeding marketability.

1. Purpose: a) Maintain/improve raw product quality of stored potatoes for sale in domestic and export markets. b) Reduce and control losses caused by damage related disease defects in stored potatoes. c) Alleviate the major impediment to marketing and profitability of potatoes in domestic and export sales. The approach includes treating potatoes with natural/endogenous regulators to determine if wound healing, "first-line defense" and elicited/signaled defense mechanisms may be enhanced to reduce disease in stored potatoes and cut seed tubers.

2. Significance: Damage related disease and defect problems impede export sales and cost the U.S. domestic potato industry close to $300 M/yr before the potato product reaches the consumer. Natural products evolving from a specific lipid metabolic system appear to be potent signaling and antimicrobial compounds that may be used to control these problems. Successful treatment with these natural products will also reduce dependence

on synthetic chemicals added to bulk stored raw potatoes to control diseases.

3. Constraints: Lack of post doctoral assistance and fiscal support for post doc.

C. Current and Future Cooperators (ARS and others):

1. Dr. Rick Bostock, UC Davis; collaborative and cooperative efforts to determine effectiveness and role(s) of lipid metabolites in controlling potato disease.
2. RRV Potato Growers Assoc.; provide field plot space.
3. ND State Univ. & Univ. of Minnesota potato breeders and pathologists provide specific genotypes for testing variations in treatment response.
4. Dr. Dennis Corsini, ARS pathologist, Idaho, provide genotypes of differing resistances.

D. Potential Uses of Research Findings:

1. Treat potatoes at harvest/during entry into bulk storage to control damage related disease problems.
2. Treat cut seed pieces to reduce disease.
3. Identification of effective natural products native to potato tuber will allow molecular amplification of endogenous expression(s) at critical time points with correct promoter.

E. Technology Transfer and End-Use Strategies and Opportunities:

If the specific lipid products/secondary metabolites provide or elicit responses that ultimately reduce disease; 1. Determine if tubers may be treated as they enter bulk storage (fall) and if added treatment (as volatiles in ventilation system) during storage (winter) provide benefit. 2. Determine if seed piece treatments (spring) may be modified to include the natural product(s) without negatively counteracting existing treatment benefits. 3. Modify expression of tuber genes endogenously generating the natural product for specific resistance <u>in situ</u>. Results in-total will be used to improve agricultural profits, provide better quality produce to processors and consumers as well as meet strict quality demands and agricultural chemical limitations imposed in exports markets. Items 1 ~ 2 ; 5-7 yrs and item 3; 5-10 yrs.

F. Thoughts on Research Needs:

Identification of mechanisms of action of effective treatments, particularly changes or activations of metabolic pathways, will facilitate and enhance effectiveness of future treatments and molecular approaches. We must not remain ignorant of how something works if we are to make future improvements after that point. Some endogenous natural products may not show great

efficacy on exogenous applications, but may be of great value *in situ* if expression is endogenously amplified via molecular techniques.

G. Chemical Methods Employed:

GC, GC-MS and HPLC for secondary product identification. Capillary electrophoresis of modified protein expressions (later in project). Fluorescent histochemical probing for deposition of specific suberin components during tuber wound healing.

H. Bioassay Methods Employed:

Determine development of infection, suberization and resistance time courses using treated intact and half tubers of specific genotypes during controlled wound healing; the wound healing tubers are challenged with bacterial or fungal rot organisms.

Name: Robert D. Lumsden

Laboratory: Biocontrol of Plant Diseases Lab.

Address: Rm 274, Bldg 011A, BARC-W
Beltsville, Md 20705

CRIS: 1275-22000-020-00D

Telephone: 301-504-5682

FAX: 301-504-5968

A. Research Accomplishments in Last 5 Years:

Developed biocontrol agents *gliocladium virens* for biocontrol of *Pythium* and *Rhizoctonia* damping-off.

Identified gliotoxin as a primary metabolite which is a component of the mechanism of action of *g. virens*.

Associated metabolites, viridin and viridiol, with biocontrol ability, but also with herbicidal action.

B. Research Objectives for Next 5 Years:

1. Purpose: Develop other biocontrol agents for disease and weed pests control. Determine mechanism of action to enhance disease development or prevent disease occurrence.
2. Significance: These will advance our ability to control plant diseases without use of synthetic chemical fungicides.
3. Constraints:(e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization) All of the above.

C. Current and Future Cooperators:

WR Grace and Co. - Co-developers of *G. virens*
Grace/Sierra Co. - Marketing organization for "Gliogard"
J.C. Locke - Florist and Nursery Crops Research Unit

D. Potential Uses of Research Findings:

Substitution or use with less amounts of chemical fungicides.

E. Technology Transfer and End-Use Strategies:

Technology transfer for gliogard started in 1986-87 and was brought to market this year 93, therefore about 6 years.

F. Thoughts on Research Needs:
G. Chemical Methods Employed:
H. Bioassay Methods Employed:

Name: Robert A. Moreau

Labortory: Plant Science and Technology Research Unit

Address: ERRC/ARS/USDA
600 East Mermaid Lane
Philadelphia, PA 19118

CRIS #: 1935-41000-031 and 1935-41000-036

Tel.#: 215-233-6428

FAX: 215-233-6559

A. Research Accomplishments (up to 5) in Last Five Years

1. Developed HPLC methodologies for the separation and analysis of lipids from plants and microbes.

2. Developed HPLC methodologies for the separation, analysis, and purification of hopanoids from bacteria.

B. Research Objectives for Next Five Years (brief description).

1. Purpose:
To investigate the possibility that hopanoids may be valuable co-products from the production of fuel ethanol by bacteria such as *Zymomonas mobilis*.

2. Significance:
Hopanoids are a recently-discovered natural product from bacteria and their potential value and usefulness to industry is unknown.

3. Constraints:
It has been difficult to develop new methods to extract and purify sufficient quantities of hopanoids in order to screen them for various biological activities.

C. Current and Future Cooperators (ARS and Others).

ARS: B. Whitaker (BARC)

University: A. Berry (UCDavis).

Industry: We recently negotiated a CRADA with Rohm and Haas Company, to evaluate the hopanoids from *Z. mobilis* for use as potential natural pesticides.

Others: National Cancer Inst. (D. Newman)

D. Potential uses of Research Findings:

Agricultural chemical uses are covered by above-mentioned CRADA. Other uses are being explored.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes);

In addition to the agricultural chemical applications covered by our current CRADA, recent preliminary results indicate that these compounds also have some interesting and unique pharmacological activities, which will provide future technology transfer opportunities.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels);

There is a need to gain a better understanding of the biosynthesis, toxicity, and potential effects of these newly-discovered compounds on the environment.

Our long-range objectives will be based on the screening results obtained during the next year.

G. Chemical Methods Employed

High Performance Liquid Chromatography
Mass Spectroscopy

H. Bioassay Methods Employed

Some of our bioassays have been performed by our industrial partner and some by a commercial contract laboratory.

Name: Joseph E. Mulrooney

Laboratory: Application Technology Research

Address: P. O. Box 350
Stoneville, MS 38776

CRIS#: 6402-21220-003-00D

Telephone No.: 601-686-5342

FAX#: 601-686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. Clarified the effects of cotton allelochemicals (gossypol, tannin, and anthocyanin) on tobacco budworm feeding and growth.

2. Determined the effect of PI, a plant growth regulator, on the allelochemical content of cotton and subsequent effects on the growth of tobacco budworm larvae.

3. Determined the effect of tobacco budworm infestation on the fruit distribution of cotton varieties infested at different developmental stages.

4. Demonstrated differences in growth rates of tobacco budworm larvae from lab populations differing in the amount of time in culture since outcrossing to the wild.

5. Evaluated the deposition and efficacy of insecticides applied by air and ground spray equipment on control of tobacco budworm and soybean looper in cotton and soybeans.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop new and innovative insecticide application methods to improve control of insect pests.

1. Purpose: The ultimate purpose of the research is to decrease insecticide application rates and the amount of insecticide moving off-target.

2. Significance: The costs for insect control to the producer and to the environment are increasing. Application methods to decrease these costs are a high priority.

3. Constraints: Aircraft and support facilities.

C. Current and Future Cooperators ARS and Others:

ARS: Kevin Howard, Lavone Lambert, and William Scott (Stoneville).

Industry: Marcus Adair and Gary Melchior (Abbott Labs).

D. Potential Uses of Research Findings:

The relationship between drop size, off-target movement, and efficacy would benefit aerial applicators and consultants. Elucidation of the mechanisms of insecticide transfer from the plant surface to the insect would aid in the development of insecticide formulations that are persistent on the plant and readily transferred to target insects. Determining the effect of aircraft design and spray-boom and nozzle type on the efficacy of insecticides would be of benefit to the chemical and application industries.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Transfer of technology through publications, cooperative research agreements, interpersonal interactions, workshops, and meetings.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

Personnel and resource expenditures for application research are very limited even though improving pesticide application is a high priority of the application and chemical industries. While the discovery of safer and more environmentally friendly pesticides is important, improving the application of those that are now on the market deserves equal attention.

G. Chemical Methods Employed:

Insecticide residue analyses by gas and liquid chromatography.

H. Bioassay Methods Employed:

Insecticide bioassays in the laboratory and field.

Name: Robert A. Norton

Laboratory: National Center for Agricultural Utilization Research, Mycotoxin Rsch. Unit

Address: 1815 N. University
Peoria, IL 61604

Cris #: 3620 42000 009D

Telephone #: 309-681-6251

FAX #: 309-681-6686

A. Research Accomplishments (up to 5) in last five years:

1. Identified 10 new metabolites in corn kernel pericarp (feruloyl and p-coumaryl sterol esters) and tested these for effect on *A. flavus* growth and aflatoxin production.

2. Identified new sterols in *Prototheca wickerhamii* and, with others, reported on several new aspects of sterol biosynthesis in algal, fungal and plant systems.

3. Identified environmental and chemical factors affecting rubber biosynthesis in guayule tissue cultures.

B. Research Objectives for next five years (brief description).

1. Purpose: A. Develop and implement assays to screen for chemical resistance in developing corn kernels to Aspergillus flavus infection and/or aflatoxin production; isolate and identify chemical factor(s) involved and determine mode of action. B. Test fractions and isolates of A. flavus cultures to identify specific compounds produced by the fungus which are involved in kernel cell death or toxicity.

2. Significance: A. Identification of specific resistance compounds could provide assays to help guide corn breeders in developing commercial corn lines with resistance to *A. flavus* infection and/or aflatoxin production. Results would allow breeders to quickly identify candidates for crossing into existing lines. B. Identification of toxic fungal metabolites and resistance to these would provide information on factors which may affect the severity of infection and the amount of aflatoxin produced by infected kernels.

3. Constraints: Limited support personnel. There is, at present, a very limited number of corn lines identified through field trials as resistant to *A. flavus* resulting in a very limited pool to work with.

C. Current and Future Cooperators (ARS and Others):

ARS: Drs. Pat Dowd, Don Wicklow, Hal Gardner, Jacob Lehrfeld and Fred Felker (N CA U R) .

Academic: Dr. W.D. Nes (Texas Tech Univ.. Lubbock. TX).

Industry: Monte Miles (Ciba-Geigy Seeds, Bloomington, IL), Dr. Jonathan Duvick (Pioneer HiBred, Int'l., Ames, IA).

D. Potential Uses of Research Findings:

Broadly, positive results could help maintain the quality safety of corn products and help maintain domestic corn and corn products in a competitive position for corn exports as world import limits on aflatoxin levels are lowered. Results could be used by commercial breeders and scientists involved in improving corn production and quality. Identification of novel anti-fungal/toxin factors in corn could suggest additional strategies to use for biodegradable fungicides. Identification of fungal metabolites toxic to corn cells could form the basis for a bioassay to screen for resistance to these factors which, in turn, could lessen the severity of *A. flavus* infection or level of toxin production.

E. Technology Transfer and End-use Strategies and Opportunities (Include predictive time frames):

Availability of a chemical marker(s) for disease resistance to *A. flavus* would allow the development of molecular probes for screening germplasm by breeders for incorporation of the trait into existing or new germlines. Positive results could be utilized by breeders immediately as a chemical screen and, perhaps, after 2-4 years as a probe screening method.

F. Thoughts on Research Needs (not being addressed in other agencies or at state level):

None.

G. Chemical Methods Employed:

Chromatography and spectroscopy of various types, including TLC, HPLC, GC, GC-MS, MS, FTIR, NMR; various types of derivatization and extraction schemes.

H. Bioassay Methods Employed:

Use of fungal cultures in vial systems of various types to test plant extracts/isolates. Use of plant cell cultures for initial screening of toxic fungal metabolites .

Name: Nichole R. O'Neill

Laboratory: Soybean and Alfalfa Research Laboratory

Address: Plant Sciences Institute
B-009, Rm 3-1
Beltsville, Maryland

CRIS #: 1275-21220-006

Telephone #: 301-504-5331

A. Research Accomplishment in Last Five Years:

Established the chronology and morphology of the expression of genetic resistance in the anthracnose disease of alfalfa, and associated several fungitoxic compounds with disease resistance.

Characterized the phenomenon of induced disease resistance and established the genetic, pathogenic, morphological, and some biochemical characteristics and requirements for induced resistance.

Investigated isolate and race pathogenic variability, correlating fungal virulence with capacity to induce resistance. Discovered two non-pathogens of alfalfa capable of inducing significant disease protection.

Developed a new, rapid, and novel extraction technique to separate phenolic and fluorescent compounds produced specifically in resistance race/clone interactions.

Determined the toxicity of induced resistance compounds (phytoalexins) to different growth stages of the pathogen.

B. Research Objectives:

The long-term objectives of this project are to elucidate and minipulate the host-pathogen interaction to improve resistance to disease.

A specific objective is to investigate the biochemical and molecular basis for induced defense gene expression in the anthracnose disease of alfalfa. Studies of induced resistance will enhance the potential for the developement of unique biocontrol technology. For example, several defense genes code for enzymes in the phenylpropanoid pathway, which lead to the production of fungitoxic defense compounds. These genes can be modified to enhance or accelerate defense gene expression. In addition to molecular manipulations and genetic engineering of the enzymes in this pathway, identification of pathogen elicitors which lead to induced resistance are potentially new classes of

biological pesticides which are more environmentally desirable.

C. Current and Future Cooperators:

ARS: C. J. Baker; N. Brooker; J. Saunders (BARC)
Non-ARS: R. Dixon and N. Paeva, Noble Foundation, Ardmore, OK; D. TeBeest, University of Arkansas

D. Potential Uses of Research Findings:

Extension service personnel, alfalfa seed producers, and forage producers will be interested in the potential for the development of new biological pesticides. Research scientists and industry will be interested in the basic research efforts involved in the identification of anti-microbial compounds and the regulation of induced disease resistance.

E. Technology Transfer and End-Use Strategies and Opportunities:

An end-use strategy will be the ability to regulate either or both of the host or pathogen gene(s) responsible for inducing disease resistance.

F. Thoughts on Research Needs:

Our biggest constraint is that we must continually find, usually outside ARS and at other locations, expertise to fill the gaps and answer research questions as they arise. The most rapid progress in the area of host-parasite interactions is being accomplished by groups of research scientists at the same location. At these locations the research performed by several scientists from different disciplines is focused by a research director. For example, to make rapid progress in my research program, we need the services, on a part-time or sometimes full-time basis, of an analytical chemist, carbohydrate chemist, molecular biologist, and mycologist. Most ARS research programs are multi-faceted and I think would be more efficiently addressed if research was conducted by coordinated efforts of a group of scientists rather than by individual scientists.

G. Chemical Methods Employed:

HPLC, TLC, column chromatography, RNA and DNA analysis. PCR

H. Bioassay Methods Employed:

Direct fungitoxicity of phytoalexin defense compounds to fungal mycelia or spores. Disease resistance assays by standard disease severity assessments.

Name: Jack Paxton

Laboratory: S-408 Turner Hall

Address: 1102 S. Goodwin
Urbana, IL, 61801

CRIS: Co-operator

Telephone: 217-333-4864

FAX: 217-244-1230

A. Research Accomplishments in Last 5 Years:

B. Research Objective for Next 5 Years:

1. Purpose: Increase soybean yields by basic understanding of biochemistry of disease resistance.

2. Significance: U.S. Soybean Crop is worth $10 billion/year, an increase of 0.1% is worth ~ $10 million/year.

3. Constraints: Fiscal, grants hard to get, directed.

C. Current and Future Cooperators:

Dr. Morris Huck Min Rhizotron, Technical support

D. Potential Uses of Research Findings:

Improved crop yields
Improved food safety
Reduced food costs
Stability in food supply
Ecologically more sustainable agriculture

E. Technology Transfer and End-Use Strategies and Opportunities:

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Names: R. G. Powell, R. J. Bartelt, R. D. Plattner, G. F. Spencer, S. F. Vaughn and R. J. Petroski

Laboratory: National Center for Agricultural Utilization Research

Address: 1815 N. University Street, Peoria, IL 61604

CRIS: 3620-22000-004-00D; 3620-42000-012-00D; 3620-41000-001-00D

Telephone: 309-681-6596

FAX: 309-681-6686

A. Research Accomplishments in Last Five Years:

1. Isolated and/or identified natural products with potential for control of insects such as the pepper weevil and several nitidulid beetles.

2. Discovered that several common constituents in foods may be used to effectively control fungal growth on fruits and berries and control sprouting of stored potatoes.

3. Identified numerous toxins in endophyte-infected grasses and suggested methods for their elimination.

4. Deeply involved in the fumonisin mycotoxin problem; structures, analysis, structure-activity relationships, detoxification and synthetic analogs.

B. Research Objectives for Next Five Years:

This research is being conducted in order to provide simple, inexpensive, and environmentally safe natural products that can be used to control insects, weeds and undesirable fungi.

1. Purpose: Protection of the food supply from insect pests, weeds and fungi utilizing products derived from nature.

2. Significance: Many of the currently used pesticides remain as undesirable residues in foods or reduce air and ground water quality. Safe, effective and environmentally acceptable alternatives are required.

3. Constraints: Problems exist in the regulatory, patenting and commercialization of natural products.

C. Current and Future Cooperators:

Cooperators include other ARS locations, other Government agencies, universities and some industries. Environmental

groups, commodity groups and smaller companies will most likely provide future support and contributions.

D. Potential Uses of Research Findings:

Safer and more selective pest control, both pre- and postharvest protection of the food supply, reduced pollution of air and water due to synthetic pesticide residues.

E. Technology Transfer and End-Use Strategies and Opportunities:

Current CRADA and Specific Cooperative Agreement should result in a formulation for monitoring and/or control of the pepper weevil within two years. With regulatory approval, natural compounds could be used to control sprouting of stored potatoes within three years. Growth of molds on harvested fruits and berries could be minimized using novel containers incorporating natural volatiles and commercialized within five years. There is evidence that certain plants can be used as green manure crops which would allow reduction of soil fumigants such as methyl bromide.

F. Thoughts on Research Needs:

Computer molecular modeling could be effectively used to design new pest control agents using natural product feedstocks. This approach is being applied with marked success in the pharmaceutical industry.

G. Chemical Methods Employed:

Finnigan TSQ-700 mass spectrometer, Bruker 400-MHz NMR, range of analytical and preparative HPLC systems, GC-MS, GC/FT-IR, etc.

H. Bioassay Methods Employed:

Diet incorporation, wind tunnel, seed germination, seedling growth, leaf discs, fungal inhibition, etc. (both in-house and through cooperators).

Name: Patricia J. W. Slininger (Spvy Chemical Engineer)

Laboratory: National Center for Agricultural Utilization Research

Address: USDA-ARS
1815 N. University St.
Peoria. Illinois 61604

CRIS #: 3620-41000-031

Telephone No.: 309-681-6286

FAX #: 309-681-6686

A. Research Accomplishments (up to 5) in Last Five Years:

1. Nutritional (C source, Fe, Zn, purines and pyrimidines) and environmental (T, pH, PO2) methods were discovered for regulating phenazine 1-carboxylic acid (PCA) accumulation in liquid cultures for producing *P. fluorescens* as a biocontrol agent against take-all of wheat.

2. Liquid culture production of a wheat seed germination inhibitor (not PCA) by *P. fluorescens* was discovered.

3. Isolation and selection technologies were developed and have led to the discovery of commercially useful bacterial antagonists of fungal potato dry rot which produce several (> 10) different antibiotics (including pyrrolnitrin identified).

B. Research Objectives for Next Five Years:

The broad CRIS objectives are a) to develop technology for mass-producing/formulating *Pseudomonas fluorescens* to control take-all of wheat (*Gaeumannomyces graminis* var. *tritici*) and b) to isolate/screen biological control agents against potato dry rot (*Fusarium sambucinum*).

PCA Impact Studies

1. Purpose: Determine how phenazine antibiotic accumulation in liquid culture productions of *P. fluorescens* for wheat seed inoculant influences seed germination, cell viability, and efficacy in controlling take-all disease (growth chamber bioassay and field trial levels).

2. Significance: Findings will influence the choice of liquid culture operating conditions and the success of formulation strategies used to mass produce and preserve viable cells for seed coatings.

3. Constraints: Growth chamber assays of stored seed inocula are time and labor intensive. Wheat seedling tube bioassays are not necessarily representative of full season field conditions, and field planting opportunities arise only twice/year.

Seed Germination Inhibitor Studies

1. Purpose: Determine the identity of and develop an assay for the wheat seed germination inhibitor produced in liquid cultures of *P. fluorescens*. Determine if inhibitor production can be controlled by appropriate choices of fermentor and or formulation conditions.

2. Significance: Seed encapsulation is a preferred method of introducing biocontrol agents into the rhizosphere because it targets emerging roots, it minimizes the amount of microbial inoculant investment needed, and it is easy for the farmer to use with existing equipment. However, detrimental effects of seed coatings on seed physiology have been observed. Our research findings are expected to yield new cell production, preservation, and formulation strategies that prevent any reduction of seed germination by bacterial coatings.

3. Constraints: Primary limitation to progress is the availability of chemistry expertise and technical support.

Novel Antibiotics

1. Purpose: Determine identities of and standard assays for antibiotics produced by bacterial antagonists of potato dry rot. Determine the roles of these antibiotics in the biological control of potato dry rot caused by *Fusarium sambucinum*. Study liquid culture production/ regulation of the antibiotics to ultimately determine how their production during liquid cultivation of the biocontrol agent influences the viability and disease-control efficacy of the resultant cell population.

2. Significance: More than three-fourths of field *F. sambucinum* strains have developed resistance to thiabendazole, the only chemical approved for postharvest protection of potatoes. Findings will influence fermentation and cell product preservation/formulation strategies that will speed commercial use of bacterial disease control agents. This research may also yield new antifungal compounds for medical or veterinary use.

3. Constraints: Due to the existence of a number of antifungal ccmpounds discovered in initial bioautography screenings, the availability of chemistry expertise and technical support are limiting factors.

C. Current and Future Cooperators and their Contributions (ARS and others)

ARS (NCAUR, Peoria, IL): R. Bothast (supvy microbiologist, RL Fermentation Biochemistry); K. Burkhead (natural products chemist); D. Schisler (plant pathologist); J. VanCauwenberge (microbiologist).

ARS (Washington State University, Pullman, WA): J. Cook (supvy plant pathologist, RL Root Disease and Biological Control Research); D. Weller (plant pathologist); L. Thomashow (geneticist).

Industry: W.Hammerbeck (United Agri Products, Greeley, CO.); D. Miller
(Ecoscience Corporation, Worcester, MA)

D. Potential Uses of Research Findings:

This research will provide discovery, fermentation, and formulation technologies which will be useful to successful mass production of microbial biocontrol agents against fungal root and tuber diseases. Natural chemical agents discovered to have possible activity in biocontrol of fungal root/tuber diseases may also have use as as antifungal antibiotics in medical or veterinary applications. Many aspects of liquid culture process technology developed for biocontrol agents would also apply to mass producing associated antibiotics for pharmaceutical use.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Technology transfer methods have included manuscripts, technical meetings and workshops (talks, posters)--for example, 1993 Beltsville Symposium XVIII (Biologically Based Pest Management), 206th ACS National Meeting (Agri- and medicinal chemicals), 77th Meeting of the Potato Association of America, 6th International Congress of Plant Pathology. When patentable discoveries are made, CRADA solicitation letters are sent to companies with potential mutual interests. One-page descriptions of new technologies are also prepared for distribution to interested visitors and contacts at meetings. In addition, participation in local and public broadcasting short documentary news releases (radio/television) helps win public support and often attracts industrial interest, especially if public broadcasting stations use these "science news briefs" nationwide. Our microbial antagonists for fungal potato dry rot control are closest to having a commercial end-use--perhaps in 5 years, IF pilot testing, scale-up, formulation, and registration go smoothly.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

From my conversations with others, I am under the impression that very little liquid culture process development is being conducted

by ARS. Such research is crucial because all microbial products/antibiotics require development of this nature in order to reach market. Fermentations are expensive processes. New economical technologies leading to product-selective fermentation processes are particularly key to the quality control and success of pricey microbe-derived pest control products in a marketplace where chemicals are relatively cheap, and field crops can range from very low to high value. After all, natural pesticides must be consistently effective and affordable relative to the crop they are expected to protect. In addition, efficient use of research time is an incentive to stimulate liquid culture rescarch. In our studies with gram-negative microorganisms for biological control, we are finding advantages in conducting studies which integrate the development of liquid culture cell production and formulation/application technologies. This approach seems to be expediting our convergence on efficacious products because we are discovering liquid culure conditions which are influencing the success of product applications.

G. Chemical Methods Employed

Standard chemical methods used include chromatoqraphy (HPLC, TLC, column LC, GC), colorimetry (W , VIS), and spectrometry (IR, NMR, mass).

H. Bioassay Methods Employed

Wounded potato bioassays were used to detect biocontrol agents against dry rot (D.A. Schisler and P.J. Slininger, submitted to Plant Disease). Natural compound activities against *Fusarium sambucinum* were assayed via TIC bioautography (K.D. Burkhead, D.A. Schisler, and P.J. Slininger, submitted to Appl. Environ. Microbiol.). Wheat seedling tube bioassays were used to assess take-all biocontrol efficacy of *P. fluorescens* seed formulations (D.M. Weller, B.-X. Zhang, and R.J. Cook. 1985. Plant Disease. 69(8):710-713; L.S. Thomashow and D.M. Weller. 1988. J. Bacteriol. 170(8):3499-3508).

Name: J. Smilanick and D. Margosan

Laboratory: USDA, ARS, Fresno, CA

Address: 2021 South Peach Ave.
Fresno, CA 93727

CRIS: 5302-43000-015-00D

Telephone: 209-453-3000

FAX: 209-453-3088

A. Research Accomplishments in Last 5 Years:

Control of brown rot of peaches/nectarines with hot ethanol.

B. Research Objectives for Next 5 Years:

1. Mode of action of hot ethanol on fungal spores
2. Most efficacious application method (tank, drench, moist air, etc.)

1. Purpose: 1-Enable modification of treatment parameters to maximize brown rot control while reducing any possible negative effects. 2-Maximize brown rot control; gain industry acceptance

2. Significance: Potential to reduce use of postharvest fungicides on stone fruit. Possibility of utilization in other commodities, e.g., citrus.

3. Constraints:
1. Air quality regulatory agencies - ethonol emissions
2. Industry acceptance; reluctance to change packing lines
3. A.T.F. regulations on use of undenatured ethanol.
4. Possible religious objection of fruit treated with alcohol. e.g., Islamic.

C. Current and Future Cooperators:

1. California Tree Fruit Agreement - Limited financial support
2. California Clean Organic Growers Organization
3. Local Growers and Packers

D. Potential Uses of Research Findings:

Stone Fruit Industry. Large scale and small scale packers and farmers. Organic farmers/packers could greatly benefit contingent upon acceptance of ethanol as "organic compound".

E. Technology Tranfer and End-Use Strategies:

Cooperation of industry during development and evaluation stages will constitute a portion of technology transfer. First commercial use may be in the organic farm industry. Providing constraints listed in #3 of survey can be overcome.

F. Thoughts on Research Needs:

Engineering and material fabrication assistance will be required.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Scientist: Dr. R. D. Stipanovic
Dr. A. A. Bell
Dr. C. R. Howell

Laboratory: Southern Crops Research Laboratory

Address: Route 5, Box 805
College Station, Texas 77845

CRIS #: 6202-22000-011-00D

Telephone #: 409-260-9232

FAX #: 409-260-9333

A. Research Accomplishments in Last Five Years:

1. Identified critical chemical and anatomical responses that occur in cotton after infection by the wilt pathogen *Verticillium dahliae*.

2. Determined the mode of action of the cotton phytoalexin desoxyhemigossypol.

3. Developed methods to control the biosynthesis of phytotoxins in the biocontrol agent *Gliocladium virens*.

4. Determined the role of secondary metabolite porduction in the biocontrol efficacy of *Gliocladium virens*.

5. Identified phytoalexins in kenaf.

Research area (3) has resulted in a successful patent.

B. Research Objectives for Next Five Years:

1. Identify and evaluate new biocontrol agents.

a. Purpose: To isolate new biocontrol agents, evaluate their effectiveness, identify new antibiotics, determine biosynthetic pathways, and augment antibiotic production.

b. Significance: This research will provide knowledge and useful materials to improve control of soilborne and seedborne diseases of cotton. These studies will facilitate the developement of new and superior strains of biocontrol agents and formulations that can be used by commercial companies and growers to treat planting seed and potentially field soil.

2. Determine mechanisms of cotton resistance to pathogens.

a. Purpose: To reduce cotton losses to plant pathognes.

b. Significance: A detailed understanding of the mechanism of resistance within *Gossypium* will facilitate improvement of resistance to various pathogens and facilitate cloning useful foreign genes and their transfer into cotton.

C. Current and Future Cooperators

Texas A&M Univ.:	Kamal El-Zik, Dept. of Soil and Crop Sciences C.R. Benedict, Dept. of Biochemistry C. W. Magill, Dept. of Plt. Pathology & Microbiology
Industry:	Gustafson, Inc.

D. Potential Uses of Research Findings

1. New Biocontrol Agents

This research will lead to superior strains of biocontrol agents, allow the development of formulations adapted to commercial use, and provide knowledge of the behavior of biocontrol agents that can be used to manage them effectively under field conditions.

2. Cotton Resistance to Pathogens

The research will identify specific beneficial secondary products within germplasm to allow cloning of genes to improve resistance to cotton pathogens.

E. Technology Transfer and End-Use Strategies and Opportunities:

1. New Biocontrol Agents

The research will be utilized by commercial companies, producers, and scientists concerned with managing cotton health, especially soilborne and seedborne diseases. Information will be useful also to scientists working on plant pathogens in other crops. Strains of *G. Virens* have been field tested. Our goal is to have a product ready for commercial use by 1997.

2. Cotton Resistance to Pathogens

The research will be used by commercial breeders, producers, and scientists concerned with improving cotton health. Our goal is to have the critical genes identified and cloned by 1998.

F. Research Needs:

Bench scientists require freedom to set long-term research goals within the mission of their Unit. While recognizing the need to rapidly transfer laboratory findings to practical applications, the Agency must continue to address long-term, high-risk research.

G. Chemical Methods Employed:

Various chromatographic techniques such as thin layer, open column, low pressure, gas, and high performance liquid chromatography are used to purify and quantitate natural products possessing unique biological activity. Chemical structures are determined using UV/visible, FT-infrared, 1D and 2D-nuclear magnetic resonance, and mass (EI, CI and FAB ionization methods) spectrometry.

H. Bioassay Methods:

Whole plant assays are used to evaluate resistance mechanisms in cotton and related genera. Petri dish assays are used to study efficacy of biocontrol organisms. A 96-well plate is used to determine toxicity of phytoalexins to pathogens and nematodes.

Names: S. F. Vaughn and G. F. Spencer

Laboratory: National Center for Agricultural Utilization Research

Address: 1815 N. University Street
Peoria, IL 61604

CRIS #: 3620-41000-001-00D

Telephone #: 309-681-6344

FAX #: 309-681-6686

A. Research Accomplishments in Last Five Years (Herbicides-Plant Growth Regulators:

1. Identified monoterpenes which have potential as parent structures for preemergence herbicides.

2. Developed synthesis of monoterpene derivatives structurally similar to the commercial herbicide cinmethylin which have activity greater than cinmethylin in laboratory tests.

3. Identified several classes of natural compounds which effectively control sprouting of stored potatoes.

B. Research Objectives for Next Five Years:

This research is being conducted in order to provide low toxicity and environmentally safe natural peoducts or derivatives which can be used to control weeds.

1. Purpose: Protection of the food supply from undesired plant growth (both in crops and weeds) utilizing products derived from nature.

2. Significance: Many of the currently used herbicides and plant growth regulators remain as undesirable residues in foods or reduce air and ground water quality. Safe, effective and environmentally acceptable alternatives are required.

3. Constraints: Problems exist in the regulatory, patenting and commercialization of natural products.

C. Current and Future Cooperators:

ARS: R. Boydston, Prosser, WA
Universities: C. Eberlein, University of Idaho; K. Al-Khatib, Washington State University, Mt. Vernon

D. Potential Uses of Research Findings:

Safer and more selective weed control, lower residues in foodstuffs and reduced pollution of air and water due to synthetic herbicide residues.

E. Technology Transfer and End-Use Strategies and Opportunities:

Certain plants which can be used as green manure crops which would allow reduction of soil fumigants such as methyl bromide and could be implemented immediately as there are no patenting or regulatory hurdles. Chemical modification of natural compounds for use as herbicides would require similar timeframes as other synthetics (5-10 years).

F. Thoughts on Research Needs:

Computer molecular modeling could be effectively used to design new herbicides using natural product feedstocks. This approach is being applied with marked success in the pharmaceutical industry.

G. Chemical Methods Employed:

Finnigan TSQ-700 mass spectrometer, Bruker 400-MH_Z NMR, range of analytical and preparative HPLC systems, GC-MS, GC/FT-IR, etc.

H. Bioassay Methods Employed:

Seed germination, seedling growth and leaf disc bioassays (both in-house and through cooperators); determinations of weed emergence in field tests of green manure crops.

Name: Charles L. Wilson

Laboratory: USDA ARS Appalachian Fruit Research Station

Address: 45 Wiltshire Road
Kearneysville, WV 25430

CRIS#: 1931-22000-005-OOD

Telephone#: 304-725-3451

Fax#: 304-728-2340

A. Research Accomplishments(up to 5) in Last Five Years:

1. Discovered, developed and tested on semi-commercial basis a number of antagonistic microorganisms for the control of postharvest diseases.

2. Development of rapid screening procedure for fungicidal activity in plant extracts.

3. Demonstrated fungicidal activity in a number of natural plant volatiles against a variety of postharvest pathogens of fruits and vegetables.

4. Demonstrated fungicidal activity in a number of plant extracts against Postharvest pathogens.

5. Developed a "bioactive coatings" for harvested commodities utilizing antagonistic microorganisms, natural compounds and resistance inducing compounds.

B. Research Objectives for Next FiveYears (brief description):

Broad objectives are to find innovative, safe and effective alternatives for synthetic fungicides to control fruit diseases. This will involve the utilization of constitutive and induced plant-derived fungicides formulated into a multifaceted biological control strategy.

1. Purpose: To reduce the synthetic pesticide load in our food supply by providing safer alternatives for the control of fruit diseases.

2. Significance: Enhanced food safety through a reduction of pesticide use on our food.

3. Constraints: Money and personnel.

C. Current and Future Cooperators

ARS: Jim Locke, Harold Moline (Beltsville, MD).

Universities: Clauzell Stevens, John Lu and Victor Kahn, Tuskegee University; Biocontrol Institute, Auburn University.

Foreign: Edo Chalutz, Volcani Institute, Israel; L. H. Cheah, Crop and Food Institute, New Zealand; Brian Wild, Greg Johnson, CSIRO, Australia; Saneya El Neshawy, Egypt; Mohamed Besri, Morocco. Regina Maria D. Gomers Carneiro, Brazil; Selma DeKoch, South Africa; and K. Azuma, Japan.

Industry: Ecogen (has licensed yeast antagonists); American Cyanamid and DowElanco [have just initiated a CRADA (BRDC agreement) to develop "bioactive coatings" for the control of postharvest diseases of fruit].

D. Potential Uses of Research Findings:

Our research is finding particular application in the control of postharvest diseases of fruits and vegetables. The major fungicides, which we have customarily depended on to control postharvest diseases, have recently been withdrawn from the market and effective alternatives do not exist. Therefore, industry has shown considerable interest in licensing and commercializing our technology. Also, some of the natural plant volatiles, which we have shown to have fungicidal activity, may be useful as an alternative to methyl bromide for the fumigation of soil.

E. Technology Transfer and End-Use Strategies and Opportunities:

Condition in the laboratory more closely approximately those found in postharvest environments than in the field. This allows a more rapid translation of laboratory results into postharvest treatments. This has been demonstrated by the fact that some of our biocontrol agents have been tested commercially only 2-3 years after they were discovered. Also, the postharvest environment has other advantages over field conditions for diseases control in that: (1) temperature and humidity are more controllable; (2) the commodity to be treated is concentrated, thereby, facilitating the application of control methods; and (3) the high value of harvested crops makes it economically feasible to apply more elaborate procedures. These advantages have been noted by industry and considerable effort is underway to commercialize this technology.

F. Thoughts on Research Needs (Not Being Addressed in Other Agencies or at State Levels):

ARS is in a unique position to: (1) develop a comprehensive data

base on bioactive natural plant compounds; (2) utilize its foreign laboratories to screen plants for bioactivity; (3) and develop cooperative screening programs with other Federal Agencies (eg. NCI) and Universities.

G. Chemical Methods Employed:

Water and organic extracts from plant sap are being screened for fungicidal activity. Unequivocal characterization of these fungicidal compounds is being done with a Gas Chromatograph equipped with a Mass Selective Spectrophotometer.

H. Bioassay Methods Employed:

A rapid method has been developed to screen for fungicidal activity in plant extracts. It employs multiwell plates and a software program which runs an ELISA plate reader that serves as a desitometer recording fungal qrowth.

Name: William T. Wilson, Ph.D.
Anita M. Collins, Ph.D.

Laboratory: USDA-ARS Honey Bee Research Unit

Address: 2413 E. Hwy. 83
Weslaco, TX 78596

CRIS # 6204-21000-004-36 (mite control)
6204-21000-005-37 (Africanized bee)

Telephone: (210)969-4870

FAX: (210) 969-4884

Research Categories: C. Natural products for parasite & pathogen control and E. Delivery systems/application technology for natural products.

A. Research Accomplishments in Past 5 Yrs.:

1. Developed and tested dosages of menthol crystals needed for fumigation and control of tracheal mites (*Acarapis woodi*) in adult honey bees (*Apis mellifera*). Now registered (EPA) for use in honey bee colonies.

2. Formic acid fumes were shown to be an effective control for the internal mite, *A. woodi*, and for the external mite, *Varroa lacobsoni*, in honey bees.

3. Field testing of menthol in a liquid carrier sprayed into the air as an aerosol to repel or discourage defensive adult Africanized honey bees from following people.

4. Field testing and employment of organic smoke and sugar syrup for reducing defensiveness in Africanized honey bees.

B. Research Objectives for Next 5 Yrs.:

The broad objective is to develop and enhance technology to improve the performance of honey bees for efficient pollination and to maximize honey production through the use of natural products.

1. <u>Control of Parasitic Mites in Honey Bees</u>. (Purpose) Continued testing of naturally occurring products for the control of parasitic mites on honey bees. (Significance) Successful mite control would result in increased honey production, better pollination and improved colony survival during winter in northern climates.

2. <u>Reducing the Level of Defensiveness in Africanized Bees</u>.

(Purpose) Further testing of natural products for repelling or calming aggressive honey bees, especially those of African ancestry. (Significance) Effective compounds would help in protecting people outdoors from possible attack by Africanized honey bees and reduce the risk of defensive bees following a beekeeper from a bee yard.

3. Constraints. Limited funds and personnel. Inadequate number of Africanized honey bee (AHB) colonies in the U.S. Inherent problems with conducting research in Mexico where many AHB colonies exist.

C. Current and Future Cooperators:

The primary research has been conducted by USDA-ARS personnel in cooperation with commercial beekeepers in the U.S. and Mexico. Informational exchange on formic acid research has taken place with personnel in the British Columbian Dept. of Agriculture in western Canada.

Texas A & M University @ Weslaco, TX: Dr. Frank Eischen.

University of Nuevo Leon @ Linares, N.L., Mexico: Dr. Celina Garza Q.

Tamaulipas Department of Agriculture: Various professional personnel including entomologists and veterinarians.

D. Potential Uses of Research Findings:

Menthol and formic acid treatments for parasitic mite control are already being used by the beekeeping industry in the U. S. and in Canada. Formic acid application does not yet have EPA approval in the U. S. but approval has been granted in Canada by the appropriate agency. The use of natural miticides will likely increase during the next 5 years. Aerosols for modifying the behavior of Africanized bees has potential value for the beekeeping industry of the U. S. and Mexico but potential use by the general public could be extensive in hot climates.

E. Technology Transfer and End-Use Strategies:

Technology transfer has already occurred with the extensive use of both menthol and formic acid by the U. S. beekeeping industry. Aerosol repellents for use in modifying Africanized bee behavior will probably be utilized and products marketed for public use within the next 5 years.

F. Thoughts on Research Needs:

Natural products appear to have a good future because they seem to be more acceptable to the general public. Some people perceive

them to be less threatening in terms of toxicity.

G. Chemical Methods Employed:

Utilized gas chromatography (GC) in the development and modification of assays for detecting residues of formic acid, menthol and amitraz. GC identification of cuticular hydrocarbons on AHB for subspecies determination.

H. Bioassay Methods Employed:

In studies with natural repellents, we measure the reduction in the number of flying bees around an experimenter (person) and the number of stings in leather targets from disturbed bee colonies. Dissection of adult bees to expose tracheal tubes for counting the number of live and dead adult *Acarapis woodi* mites.

Name: Ida E. Yates

Laboratory: Pecan Natural Products

Address: Microbial Products Research Units
P.O. Box 5677, RRC, ARS, USDA
Athens, GA 30613

CRIS #: 6612-22410-002-00D

Telephone No.: (706) 546-3523

FAX #: (706) 546-3250

A. Research Accomplishments (up to 5) in Last Five Years:

1. Co-operated with entomologists, pathologists, horticulturalists, and chemists to design and execute experiments discrediting the long-held concept that in vivo levels of juglone conferred scab resistance to particular pecan genotypes
2. Markers identified as selection tools for genotypes with reduced susceptibility to sooty mold.
3. Demonstrated practical applications of stored pecan pollen for field use.
4. Regenerated pecan plants from plant tissue culture.
5. Instrumental in the development of gene transformation in pecan providing unprecedented potential for genetic improvements in pecan.

B. Research Objectives for Next Five years (brief description; also fill in attached table using brief descriptors):

Natural Products Enhancing Crop Efficiency in Pecan

1. Purpose: Determine the target sites and natural products inherent to the pecan germplasm which control bioregulatory processes conferring characteristics beneficial for low input, biosafe crop production with potential application to other important horticultural and agronomic crops.

2. Significance: The results are expected to result ultimately in the development of utilitarian products for conserving an environmentally safe biosphere, while maintaining agriculture as a financially viable enterprise in the United States.

3. Constraints: Fiscal support.

C. Current and Future Cooperators and their Contributions (ARS and others):

- Drs. D. Sparks and T. Crocker, Dept. Horticulture, UGA - Pecan germplasm resources.

- Drs. C. Bacon, C. Mims, and C. Reilley - RRC, ARS, USDA, Mycotoxin Research Unit; Dept. Plant Pathology, UGA; and Southeastern Fruit and Tree Nut Laboratory, Byron, GA, respectively - Fungal metabolism.
- Louis Tedders - Southeastern Fruit and Tree Nut Laboratory, Byron, GA, - Insect physiology.
- Phytochemical Research Unit, RRC, USDA, ARS - Chemical analyses.

D. Potential Uses of Research Findings:

Results will contribute to the basic knowledge in growth and development of nut crops, specifically pecans, and woody plants, in general. Results will have application to the tree nut industry by providing natural products that are biosafe and eventually, novel germplasm and/or the information and technology for altering existing germplasm to enhance crop efficiency in these economically important plants.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Time frames):

The possibility to stipulate success and predict time tables in the solution of problems of such difficulty and magnitude is not possible even though a reasonable expectation of fruitful work is presupposed.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Pecan is unique among economically important crops in having native populations in the United States which creates an extensive gene pool for identifying and selecting beneficial crop traits. Genetic variability has been identified for more efficient crop production traits including diversity in reproduction patterns and resistance to pests. However, efforts to utilize the natural products inherent to this germplasm has been neglected. The solution of these problems would represent major advances of great significance opening the way for extensive related development in other important crops.

G. Chemical Methods Employed: HPLC, GLC, MS, GC, 2-D polyacrylamide electrophoresis and other macromolecular techniques.

H. Bioassay Methods Employed: Germ tube and appressorium formation on a range of biotic and abiotic substrates, infection of biotic substrates.

Natural Products for Weed Control

* Indicates that the information is also relevant to other research areas and has been included as such.

*

Name: Hamed K. Abbas

Laboratory: Southern Weed Science Laboratory

Address: P.O. Box 350
Stoneville, MS 38776

CRIS #: 6402-22000-012-00D

Telephone #: (601) 686-5313

FAX #: (601) 686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. A method of isolation and purification of the natural products AAL-toxin and its analogues was developed.
2. Many natural products were tested for phytotoxicity on intact plants, duckweed, excised leaves and tissue culture.
3. A method was developed for biosynthesis of hydrolysis products of the fumonisins and AAL-toxin.
4. AAL-toxin, diacetoxyscirpenol and T-2 toxin were shown to have insecticidal activity against tobacco budworm larvae (*Heliothis virescens*).
5. AAL-toxin was patented (U.S. Patent # 5,256,628) as a herbicide on October 26, 1993.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: The purpose of the overall objective is to identify and evaluate natural products that have potential uses as bioherbicides. This includes: a) development of analogues of AAL-toxin and fumonisins, and b) isolation and identification of new phytotoxins from pathogenic and non-pathogenic fungi. Radicinin (isolated from *Alternaria helianthi*), tenuazonic acid (isolated from *A. alternata*), Fusaric acid and moniliformin (isolated from *Fusarium* spp.) and unidentified phytotoxin appear to have potential in preliminary tests.

2. Significance: Herbicides derived from biological sources often have unique mechanisms of action. They have the potential for replacing current herbicides with more toxicity or that are losing effectiveness because of development of resistance. Natural products tend to be safer for the environment and more biodegradable than conventional herbicides.

3. Constraints: Fumonisin has been reported to cause mammalian

toxicity, which has hindered research on applications for weed control. The research situation including technical help and funding is unstable.

C. Current and Future Cooperators (ARS and Others):

ARS: Stephen O. Duke, Joseph E. Mulrooney, James E. Hanks, Rex N. Paul (Stoneville, MS).

FS: W.T. Shier, T. Krick, C.J. Mirocha (U. Minn.), J. Kuti (Tex. A&I U., Kingsville, TX), G. Kraus (Iowa State U., Ames, IA).

Industry: Luke Lam (LKT Laboratories, Inc., St. Paul, MN).

D. Potential Uses of Research Findings:

Development of fungi and the natural products as herbicides for weed control and determination of mechanism of action of these herbicides. Industry is interested in commercial applications.

E. Technology Transfer and End-Use Strategies and Opportunities:

AAL-toxin has been patented as a herbicide (U.S. Patent No. 5,256,628 on October 26, 1993). It and its analogues should be of interest to the herbicide industry within the near future. Other products will be marketed to industry as they become available.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

We need cooperation with others in the USDA working on similar projects. It would be helpful to have those working on natural products for weed control at one location. Stability in funding would help to ensure that research objectives could be carried out.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Intact plants
Duck weed
Excised leaves
Tissue culture
Heliothis virescens

*

Name: Stephen Beckstrom-Sternberr
James A. Duke

Laboratory: National Germplasma Resource Laboratory

Address: USDA, ARS, B-003 R-227, BARC-West
Beltsville, MD 20705

CRIS: 275-21000-057-00D

Telephone: 301-504-5419

FAX: 301-504-5536

A. Research Accomplishments in Last 5 Years:

Natural Pesticide Database Developed

B. Research Objectives for Next 5 Years:

1. Purpose: To discover or popularize natural pesticide as alternative crop to protectants.
2. Significance: Natural pesticides and medicinal compounds are becoming more popular with consumers and USDA should be more consumer-driven.
3. Constraints: Political, Security. (in foreign locations)

C. Current and Future Cooperators and their Contributions:

Weed Science Laboratory (USDA) (Growing Greenhouse Crops) CRC Press (published database)

D. Potential Uses of Research Findings:

Crop diversification in the humid tropics.
Reduction in narcotic exports from Peru

E. Technology Transfer and End-Use Strategies:

Trying, with civilian conterparts, also to develop marketable alternative crops, including pesticides, medicine, hypoallergenic rubber, low fat chocolate, antioxidant salad dressings.

F. Thoughts on Research Needs:
Critical evolution would favor synergism between pesticidal compound, in plants. Yes, USDA and Industry go for the silver bullet.

G. Chemical Methods Employed:
H. Bioassay Methods Employed:

*

Name: John M. Bland

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687,New Orleans, LA 70179

CRIS #: 6435-41000-018-00D

Telephone No.: (504) 286-4279

FAX #: (504) 286-4367

A. Research Accomplishments in Last Five Years:

1. First Synthesis of the natural fungicide, iturin.
2. New synthesis of B-amino acids/synthesis of iturinic acid.
3. Synthesis of iturin analogs for SAR studies.
4. Synthesis of tentoxin analogs for SAR studies.
5. New synthesis of the natural herbicide, tentoxin.

B. Research Objectives for Next Five years:

1. Purpose: To determine the structural and spatial components of: A) Iturin and B) Tentoxin, needed for biological activity. Produce an economically feasible A) fungicide, B) herbicide with the environmentally-safe properties of the parent compound.
2. Significance: Development into a viable replacement for the commercially available fungicides being removed from the market because of their toxic residues on foods and contamination of ground water.
3. Constraints: Manpower

D. Potential Uses of Research Findings:

New commercial products:
A) Fungicide B) Herbicide

G. Chemical Methods Employed:

Solution and solid phase peptide synthesis methods.

H. Bioassay Methods Employed:

A) Fungal inhibition zone on agar plate.
B) Lettuce seedling chlorosis.

Name: Rick Boydston

Laboratory: Irrigated Agriculture Research and Extension

Address: Rt. 2 Box 2953-A
Prosser, WA 99350

CRIS: 5354-13220-001-00D

Telephone: 509-786-2226

FAX: 509-786-4635

A. Research Accomplishments in Last 5 Years:

Demonstrated weed control benefits in potatoes using green manure crops of *Sinapsis alba* and *Brassica napus*.

B. Research Objectives for Next 5 Years:

1. Purpose: Develop green manure crop management in potatoes to control weeds and determine mechanism of activity of green manure crops on weeds.

2. Significance: Could allow growers to use less herbicide inputs in potato production.

3. Constraints:

C. Current and Future Cooperators:

Steven Vaughn, Peoria, IL, ARS, Identify breakdown products of green manure crops and their activity on weeds.

Charlette Eberlien, Weed Scientist, Aberdeen, ID, Univ. of Idaho.
Kassim Al-Khatib, Weed Scientist, Mt. Vernon, WA, Wash. St. Univ.
Scott Nissan, Weed Scientist, Lincoln, NE, Univ. of Nebraska.
(Identify activity of green manure crops in weeds and how to manage for optimum weed control.

D. Potential Uses of Research Findings:

Control of weeds with less herbicide inputs in potatoes and other crops.

E. Technology Transfer and End-Use Strategies:

- Educate growers on green manure crop benefits for weed, nematode, and disease control. Educate growers on how to manage green manure crops for best results.

- Field days and state meetings.

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

*

Name: Horace G. Cutler

Laboratory: Microbial Products Research Unit

Address: Richard B. Russell Research Center
P.O. Box 5677
Athens, GA 30613

CRIS No.: 6612-41000-001-00D

Telephone No.: (706) 546-3378

FAX No.: (706) 546-3250

A. Research Accomplishments in Last Five Years:

1. Isolation and identification of a natural product fungicide. The material is presently in field trials to control *Armillaria* in Kiwifurit and *Pinus radiata* and to control silverleaf in Asian pears and ornamentals. It is biodegradable and safe.

2. Isolation of a bioremediating organism that aerobically breaks down PCPs (under patent).

3. Isolation and identification of (-) harzianopyridone, a fungal natural product with herbicidal properties.

4. Isolation of botcinolide, a novel natural product herbicide.

5. Antimicrobial and plant growth regulating properties of sucrose esters.

B. Research Objectives for Next Five years:

The objective is to isolate, characterize, and utilize biodegradable natural products from microorganisms for agricultural and other uses.

1. Purpose: To isolate, identify and use as herbicides, antimicrobials/antivirals, natural products from microorganisms for practical use as biodegradable chemicals (environmentally benign agents) and/or pharmaceuticals.

2. Significance: To protect the enviroment, to produce quality pesticide free food and to produce value-added products from fermentation.

3. Constraints: Technical - Need NMR facilities and easy

access to X-ray crystallography. Need FAB-MS and HRP-MS.

C. Current & Future Cooperators:

- Dr. Robert A. Hill, HortResearch, New Zealand. Microorganisms and field tests. $25,000
- Dr. L. Cheah, Food Research Ltd, New Zealand. Post harvest applications of natural products.
- Dr. H. Rhothitha, HortResearch, New Zealand. Entomology
- Dr. Karst Hoogsteen, Merck Therapeutic Research. X-ray crystallography.
- Dr. Gary Newton, Univ. of Georgia, X-ray crystallography
- Dr. Stephen Cutler, College of Pharmacy, Mercer Univ. Chemical Synthesis
- Dr. John Jacyno, College of Pharmacy, Ohio Northern Univ. Toxicology.

D. Potential Uses of Research Findings:

Biodegradable, environmentally safe agricultural chemicals for uses both pre and post harvest to protect crops and products. These will protect consumer health and will protect the export market. In addition, we expect financial profit from these developments.

E. Technology Transfer and End-Use Strategies and Opportunities:

To industry, for development, either before or after the patent process. Some of our materials are already undergoing field trials (Timeframe: now and < 10 years).

F. Thoughts on Research Needs:

There are several natural product sources that are not being tapped because of lack of financial support and, especially, a lack of imagination in spending and <u>obtaining</u> funds.

G. Chemical Methods Employed:

Column chromatography, Prep HPLC, HPLC, TLC, Spinning Plate, UV, FT, R, 1HNMR, ^{13}CNMR, LRP_{MS}, FABMS, X-ray crystallography.

H. Bioassay Methods Employed:

Etiolated wheat coleoptile bioassay; phytotoxicity tests on greenhouse-grown plants; antibacterial and antifungal bioassays; antitumor bioassays (extra-mural).

Name: Stephen O. Duke

Laboratory: Southern Weed Science Laboratory

Address: P. O. Box 350
Stoneville, MS 38776

CRIS #: 6402-22000-011-OOD

Telephone No.: 601-686-5272

FAX #: 601-686-5422

A. Research Accomplishments with Natural Products in Last Five Years:

- Determined physiological effects of colletotrichin
- Determined mode of action of cornexistin
- Helped to determine mode of action of fumonisins and AAL-toxin
- Localized artemisinin production in wormwood
- Determined a molecular site of action of cyperin
- Correlated physiological effects of tentoxin
- Bioassayed a large number of fungal toxins for phytotoxicity
- Evaluated the potential of natural porphyric herbicides

B. Research Objectives for Next Five Years:

- To evaluate the herbicidal activity of vulgarin and possibly determine its mode of action
- To evaluate the herbicidal activity of selected secondary marine products
- To do structure/activity relationship research on AAL-toxin-related phytotoxins
- To further evaluate the mode of action of AAL-toxin type phytotoxins
- To further examine the mode of action of cyperin
- To evaluate any other natural products that look interesting

C. Current and Future Cooperators on Natural Product Research:

Jim McChesney, Mark Hamann, and Hala ElSohly at the Univ. of Mississippi
George Sturtz, AromaGen, Albany, OR
Ron Riley, ARS, Athens, GA
David Gealy, ARS, Stuttgart, AR
John Lydon, ARS, Beltsville, MD
Gary Elzen, ARS, Stoneville, MS

D. Potential Uses of Research Findings:

1. Supporting data for ARS patents on natural product-derived herbicides
2. Supporting data to evaluate potential toxicity
3. Supporting data to interest possible industry licensees

E. Technology Transfer and End-Use Strategies and Opportunities:

The ultimate aim is for natural products from my program to be developed as commercial herbicides. If we are successful, it will take a minimum of 4 to 5 years.

F. Thoughts on Research Needs:

We need to have a critical mass of scientists focused on natural products in one place under one leader who can motivate and coordinate the group. Our current efforts are fragmented, and in many instances the ARS scientists involved in this research spend only a small fraction of their time on it.

G. Chemical Methods Employed:

HPLC, spectrofluormetry, spectrophotometry, TLC, PAGE

H. Bioassay Method Employed:

Duckweed, lettuce seedling, whole plant, leaf discs, and many others, depending on the requirements.

Name: David R. Gealy

Laboratory: Rice Production and Weed Control Research

Address: Rice Research and Extension Center,
Box 287 Hwy. 130 East
Stuttgart, AR 72160

CRIS#: 6225-22000-002

Telephone: 501-673-2661

FAX: 501-673-4315

A. Research Accomplishments in Last Five Years:

1. Cell-free supernatant and semipurified precipitate from *Pseudomonas fluorescens*-D-7: a) inhibit growth of *Bromus tectorum* roots by inhibiting lipid synthesis and disrupting cell membranes; b) contain a complex of peptides, chromopeptides, and a lipopolysaccharide matrix, which collectively are required for root inhibition.
2. Ethylacetate extracts of *Pseudomonas syringae*-3366: a) inhibit growth of *B. tectorum* and several other weed and crop species in agar and soil bioassays; b) are stable in dry soil for 6 months; c) contain several biologically active compounds including phenazine dicarboxylic acid and aminophenoxazone.

B. Research Objectives for the Next Five Years:

1) Purpose: a) Screen for herbicidal compounds in extracts of additional microbial cultures; b) isolate and chemically and physiologically characterize active compounds; c) discover chemical and physiological nature of allelopathic compounds produced by weed-suppressing rice cultivars.

2) Significance: Develop alternate methods to manage weeds.

3) Constraints: Few individuals with appropriate background, inadequate laboratory facilities, lack of enough positive results to get industry seriously interested.

C. Current and Future Cooperators:

1) S. Gurusiddaiah, Alex Ogg, Jr., Frank Young, Ann Kennedy, Pullman, WA.
2) R. Dilday, N. Rutger, T. Lavy, Stuttgart and Fayetteville, AR.
3) S. Duke, R. Hoagland, and others at Stoneville, MS.
4) Several developing relationships with industry.

D. Potential Uses of Research Findings:

Discovery of naturally existing chemistries and/or natural products to control weeds.

E. Technology Transfer and End-Use Strategies and Opportunities:

We are many years away from effective technology transfer or end use activities.

F. Thoughts on Research Needs:

G. Chemical Methods Used.

H. Bioassay Methods Employed:

1) Seed germination on agar plates, on soil plates, and in field soils.

2) Root growth inhibition in hydroponics.

*

Name: Howard Harrison, Joseph Peterson

Laboratory: U.S. Vegetable Laboratory

Address: 2875 Savannah Hwy.
Charleston, SC 29414

CRIS #: 401-6659-050-05 and 0

Telephone #: 803-556-0840

FAX #: 803-763-7013

A: Research Accomplishments in Last Five Years:

1. Demonstrated that sweetpotato interference with yellow nutsedge and other weeds is due in part to allelopathy.

2. Isolated a sweetpotato root periderm compound that is highly inhibitory to yellow nutsedge root growth and velvetleaf and proso millet seed germination. Selectivity between species in seed germination inhibition was also observed.

3. Field and greenhouse studies indicated that the growth of allelopathic sweetpotato clones were reduced less by weed interference than non-allelopathic clones.

4. Demonstrated yellow squash components are highly inhibitory to proso millet seed germination and may contribute to its allelopathic properties.

B. Research Objectives for the Next Five Years.

1. Describe traits (allelopathic and morphological) that give sweetpotatoes greater competitiveness against weeds.

2. Isolate for identification by cooperating chemists other allelochemicals from sweetpotato.

3. Isolate for identification by cooperating chemists allelochemicals from squash.

4. Develop laboratory techniques to identify pest resistant and allelopathic crop genotypes.

C. Current and Future Cooperators.

ARS; P. Dukes (Pathologist), J. Bohac (Geneticist), J. Thies (Nematologist)

USVL, B. Horvat (Chemist), J.K. Porter (Chemist) Athens, W. Lusby (Chemist) Beltsville.
University; M. Sheppard (Entomologist), Clemson., D. LaBonte (geneticist),
C. Motsenbacher (Weed Scientist), LSU.

D. Potential Uses of Research Findings.

This research is part of a multidisciplinary, collaborative effort with the broad objective of investigating the biochemical bases for host plant resistance in vegetable crop species and isolating and identifying compounds present in vegetable crops that are responsible for allelopathy and disease and insect resistances. Potential benefits of this research are; (1) discovery of new natural products that may be useful for pest control, (2) development of "in vitro" methods to identify resistant or allelopathic crop genotypes, (3) development of bioassays for identifying pesticidal natural products or resistant plant genotypes, (4) provide information useful in identifying "resistance genes" that can be transferred to other plants by conventional or biotechnological methods.

E. Technology Transfer and End Use Strategies and Opportunities.

Several agrichemical companies have expressed interest in investigating sweetpotato defense compounds as natural products for pest control; however, no formal agreements have been made. The most likely end use of this research is identification of constituents in vegetables that contribute to pest resistances. This will allow plant breeders to quickly quantify pest resistance levels, a process which now takes several years. The first strategy is long term and would probably take at least 10 years to reach the market. The second strategy could be implemented as soon as methods to quantify resistance factors are developed.

F. Thoughts on Research Needs (not being addressed in other Agencies or at the state level).

There is a wealth of information known to subsistence farmers in undeveloped areas about the use of living or dead plants to suppress weeds and other pests. A concerted effort to study these practices and to identify and preserve the species used could provide leads toward the identification of pesticidal natural products and species useful as cover crops for pest suppression in an IPM program.

G. Chemical methodologies in Use.

HPLC, GC, column chromatography, TLC.

H. Bioassays in Use:

Various seed germination tests, yellow and purple nutsedge root growth, *Fusarium oxysporum* mycelial growth, root knot nematode egg hatching and survival, diamondback moth and fall armyworm growth and survival.

*

Name: Robert E. Hoagland

Laboratory: USDA/ARS/Southern Weed Science Laboratory

Address: P.O. Box 350
Stoneville, MS 38776

CRIS #: 6402-22000-012-00D

Telephone #: 601/686-5210

FAX #: 601/686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. Developed a hydroponic seedling bioassay system to measure efficacy of pathogens/phytotoxins on weeds.

2. Found phenylalanine ammonia-lyase activity and phenolic metabolism was involved inweed defense against two important weed pathogens, indicating the action of microbial elicitors and/or phytotoxins.

3. Found (with cooperators) a fungal isolate on diseased jimsonweed that produced high amounts of phytotoxin, later identified as fumonisin. Examined the selectivity and action of this compound in various weed and crop plants.

4. Found that several natural products with anti-bacterial activity also have selective phytotoxic activity.

B. Research Objectives for Next Five Years (brief description):

a. Investigate chemical synthetic and natural product interactions with weed pathogens in order to increase or regulate efficacy and/or host ranges.

b. Examine physiological/biochemical effects of phytotoxins produced by microbes.

1. Purpose: To increase microbial pathogen efficacy, alter or regulate plant host range and to provide basic knowledge about the action of these compounds in plants.

2. Significance: Information will be valuable in the development of new and more environmentally safe natural and/or synthetic herbicides.
3. Constraints: Technically, this research is very long-term.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Cooperators include: ARS, industrial and university scientists.

Contributions of cooperators include: Supply of cultures of potential pathogens for weeds; supply of formulations that promote infectivity; field testing of important laboratory findings.

D. Potential Uses of Research Findings:

Discovery of novel phytotoxins useful as herbicides or herbicide development.

Better understanding of the physiology and biochemistry of pathogen/plant interactions and phytotoxin action.

Regulation manipulation of host range of pathogens for weeds.

E. Technology Transfer and End-Use Strategies and Opportunities:

Basic scientific information is published in journals, reviews and/or books. Some novel microbe-plant-chemical interactions that may have patent potential will be transferred to industrial or academic cooperators. This may then lead to product development useful for weed control.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Funding stability is needed to meet the research goals.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Name: Robert J. Kremer

Laboratory: USDA-ARS-CSWQRU

Address: 138 Mumford Hall
University of Missouri
Columbia, MO 65211

CRIS: 3622-22000-014-00D

Telephone No.: 314-882-6408

FAX No.: 314-884-4960

A. Research Accomplishments in Last Five Years:

1. Described association of deleterious rhizobacteria on weed seedlings and their potential as biocontrol agents of weeds.

2. Described use of deleterious rhizobacteria combined with low rates of chemicals to enhance biocontrol activity toward seeds and seedlings in soil.

3. Developed rapid screening bioassays for efficient detection of phytotoxin-producing bacteria and fungi detrimental to several weed species.

4. Using tissue culture assays, discovered microorganisms associated with leafy spurge that inhibit plant growth via production of cell-free phytotoxins.

B. Research Objectives for Next Five Years:

1. Microorganisms for Biocontrol of Perennial Weeds:

a. Purpose: Discover microorganisms for biocontrol of weeds regenerating from vegetative structures.

b. Significance: Perennial weeds are highly competitive, difficult to control, and are increasingly becoming problems in no-till crop production. New agents are needed to attack and deliver phytotoxic metabolites to the plant at early, vulnerable stages--unlike mycoherbicides in current development.

c. Constraints: Obtaining adequate technical assistance to initiate the project.

2. Bioherbicidal Activity of Microbial Isolates:

a. Purpose: Evaluate microbial culture collection for isolates with bioherbicidal activity.

b. Significance: Detection of "natural products" with bioherbicide activity will a) provide information on mechanisms of action by deleterious rhizobacteria; b) reveal potential for development of useful bioherbicides; and c) provide potential alternatives for reducing use of chemical herbicides.

c. Constraints: Obtaining and funding adequate technical assistance to conduct research.

3. Integrated Biocontrol Strategies:

a. Purpose: evaluate combination of two or more compatible agents for improved control of weeds.

b. Significance: Integration of two or more effective agents (which may or may not product phytotoxins) can hasten the demise of target weeds thereby reducing length of competition with crop and eliminating production of propagules by the weed.

c. Constraints: Technical assistance for field testing; potential regulatory obstacles for field releases.

4. Soil-Applied Biocontrol Agents:

a. Purpose: Enhance growth/activity (metabolite synthesis) by selected biocontrol agents applied to soil.

b. Significance: Natural product activity may only occur when microbe is present at the target site (i.e., rhizosphere). Thus, to insure adequate numbers of agent in environment, select agent with ability to establish and compete with native microbes (i.e., utilization of exotic C/N sources, antibiotic production).

c. Constraints: Availability of competent personnel to perform work; potential patent, regulatory issues.

5. Formulations for Biocontrol Agents:

a. Purpose: Develop effective, practical formulations for delivery of biocontrol agents.

b. Significance: Formulations that sustain bioactivity of agents and/or their natural products must be developed for use in the field and to maintain effectiveness under a range of environments.

c. Constraints: Possible technical aspects if formulations already available cannot be adapted.

C. Current and Future Cooperators and Their Contributions:

USDA-ARS: Ann Kennedy, Tony Caesar, Neal Spencer
New Jersey Dept. of Agriculture: Dan Palmer
University of Missouri: Muhammad Sarwar
University of Minnesota: Don Wyse

D. Potential Uses of Research Findings:

Biological control options for control of annual and perennial weeds in row crops and pastures.
Basic understanding of modes/mechanisms of action by certain microbial biocontrol agents.
Synthesis of new weed control chemicals based on natural products (microbial metabolites).

E. Technology Transfer and End-Use Strategies:

F. Thoughts on Research Needs:

Little effort toward research on integrating biological control or natural products with conventional practices.
More focus on ways to manipulate weed propagules in soil to include biocontrol agents and natural products.
Need to concentrate on a highly visible weedy pest for control with biological control or natural products to obtain a "success story" causing public attention and subsequent support for such programs.

G. Chemical Methodologies in Use:

UV/VIS Spectrophotometry, TLC, Paper Chromatography, HPLC

H. Bioassays in Use:

Lettuce seedling bioassay; Direct bioassays on selected weed seedings; Host-Pathogen Interaction System (HPIS) for tissue culture

*

Name: Alan R. Lax

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-018-OOD

Telephone No.: (504) 286-4382

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Demonstrated Iturin A to be active against mycotorganic organisms.

2. Discovered genomic DNA homologous with known cyclic peptide synthetases in *Bacillus subtilis*.

B. Research Obiectives for Next Five Years:

1. Purpose: Increase iturin protection to commercially acceptable levels.

2. Significance: Provide safe and effective fungal control agents for crops and commodities.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):

   Technical - Increase production. Patent - Iturin has long been known as a antifungal agent.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Gustafson, Inc. Dallas, Texas: fermentation (large scale); strains of producing organisms.

D. Potential Uses of Research Findinqs:

Provide safe (non-carcinogenic, fungal specific) fungicide in economically significant quantities.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Strategies for iturin use include fermentation of engineered organisms for increased production of the peptide and use of that product as a traditional fungicide. The anticipated timeframe for engineering organisms for increased production is predicted to be two or three years. Additional strategies to incorporate the biosynthetic genes for iturin(s) into crops to produce their own fungicides are anticipated to begin upon successful cloning of the biosynthetic genes and are expected to be completed over a much longer timeframe. Fermentation industry and seed companies are the projected beneficiaries of technology transfer. Ultimately savings are expected through reduced costs of application and broader control for the grower.

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

Name: John Lydon

Laboratory: Weed Science Laboratory

Address: Bldg. 001 Room 236
BARC-W Beltsville. MD 20705

CRIS #: 1275-22000-087-OOD

Telephone #: 301-504-5379

FAX #: 301-504-6491

A. Research Accomplishments Related to Natural Products in Last Five Years:

1. I and coworkers developed a tissue culture procedure to study the production of alkaloids in *Erythroxylon coca* var. coca. The procedure was used to demonstrate that the non-selective herbicide glyphosate blocks cocaine production in this system.

B. Research Obiectives for Next Five Years:

1. a. Purpose: Develop biological control agents for annual weeds in annual crops. Emphasis is on altering presently developed or potential mycoherbicdes with the use of recombinant DNA technologies to increase efficacy and expand host range to include related weed species.

   b. Discover herbicidal compounds from plants and microorganisms and determine specificity and mode-of-action.

2. Significance: The discovery and development of natural control agents for weeds will result in less dependence upon synthetic herbicides.

3. Constraints: The use of transformed plant pathogens requires use of special containment facility.

C. Current and Future Cooperators (ARS and Others):

ARS: C.F. Mischke, N.L. Brooker (BARC); W.L. Bruckart, (Frederick, MD); A.C. Kennedy, (Pullman, WA).

Non-ARS: N. Toscano (U. Calif., Riverside); M. Abou-Donia (Duke U., Durham, NC).

D. Potential Uses of Research Findinqs:

Improved mycoherbicides and new natural herbicides will be made available to industry through CRADAs for further

development into products for agriculture.

E. Technology Transfer and End Use:

If results are promising with natural products being investigated, industry will be contacted to determine the commercial potential as herbicides. In the work on transforming microbes to produce phytotoxins to improve their potential as bioherbicides, the package will be patented and offered to industry should it prove to be an improved biocontrol method.

F. Thoughts on Research Needs:

Negative results are often not published. However, such information could save a lot of time researching materials/compounds that prove to be inactive. The data base discussed at the workshop would help prevent this problem. In addition, another item discussed at the workshop was a directory of companies interested in natural products as pesticides who would be interested in forming CRADAs. The list should include the name of a company representative to whom inquires could be made. It was suggested that someone in Technology Transfer could but this data base together. I think it would be very valuable.

G. Chemical Methodologies in Use:

Regarding our work with microbial broths, most of the compounds that we are investigating are very water soluble. Therefore, we rely mainly on ion-exchange chromatography in the isolation and purification of the supernatant. Where plant materials are investigated, materials are first extracted with a non-polar solvent, then with a polar solvent, and finally with water. The approach used to further fractionate the extracts is dependent upon which of the original extracts were active. It may involve liquid/liquid extraction, column chromatography, thin-layer chromatography, etc.

H. Bioassays in Use:

Lettuce seed germination and radical growth and the Lema bioassay are used to assess herbicidal activity. An *E.coli* bioassay is often used as a quick assay for following the active constituent(s) through the extraction process.

Name: Paul H. Orr

Laboratory: Potato Research Laboratory

Address: 311 5th Avenue N.E.
P.O. Box 113
East Grand Forks, Minnesota 56721

CRIS #: 3650-43000-004

Telephone #: 218/773-2473

Fax #: 218/773-2207

A. Research Accomplishments in Last Five Years:

1. Designed and assembled a series of 216 glass environment chambers (20-lb cap.) each provided with its own regulated air supply and humidification via a distribution system consisting of air and water pipes, valves, nozzles and critical orifices. The chambers are capable of simulating atmospheric conditions encountered in a large potato storage bin.

2. Certain natural products were eliminated and others retained as good potential sprout control substances for possible commercial application.

3. Research has resulted in one patent application and another potential patent application.

B. Research Objective for Next Five Years:

Evaluate natural substances for use as sprout control agents in stored potatoes.

1. Purpose: Determine the sprout suppression properties of selected natural products with regulatory or allelochemical properties in test apparatus that simulates potato storage environmental conditions.

2. Significance: Global and U.S. industries are demanding that new effective natural sprout control technology be developed to replace the forty year old technology of synthetic sprout inhibition presently used on stored potatoes; sprout control is essential in maintaining quality of stored potatoes.

3. Constraints: Step-by-step testing requirements toward scale-up and seasonality of crop create timeframe constraints. Commercialization requires technology transfer, via CRADA's, etc., but industry cautious due to high cost of development. Ultimately all regulatory requirements for application to food

products must be met. Require added trained support help. Need postdoctoral help to determine biological mechanism of action of natural products currently undergoing patent application.

C. Current and Future Cooperators (ARS and Others):

- Dr. Edward C. Lulai, ARS, Potato Research Laboratory, East Grand Forks, MN provides biochemical expertise.
- Martin T. Glynn, Research Assistant, North Dakota State Univ., Potato Research Laboratory, East Grand Forks, MN provides technical expertise for daily operation of project.
- Jerry M. Sacks, formerly ARS, provided statistical expertise. Red River Valley Potato Growers Association, East Grand Forks,MN produces the potatoes for the tests. National Potato Council recommends allocations of funds to the project.
- Dr. Galen Kleinkopf, U. of Idaho, exchanges data and their own testing information, will determine residue analyses protocols.
- Dr. Jeffrey C. Suttle, ARS, Plant Physiologist, Fargo, ND exchanges information and expertise on dormancy as a means of sprout suppression.
- Dr. Steven Vaughn, ARS, NCAUR, Peoria, IL initial test of some compounds.

D. Potential Uses of Research Findings:

Sprout control is an essential component of quality maintenance in commercially stored potatoes. New technology would replace the current aging synthetic sprout inhibitor technology. Technology to naturally control sprouting will benefit growers and processors requiring long-term raw product storage as well as meet emerging export demands. A safe and natural means of controlling sprouting may alleviate consumer safety concerns.

E. Technology Transfer and End-Use Strategies and Opportunities:

F. Thoughts on Research Needs:

A considerable amount of detailed research is required to identify biological effects, residues, metabolic pathways, application techniques, timing, etc. of any natural compounds exhibiting potential as sprout suppressants.

G. Chemical Methodologies in Use:

After treatment, gas chromatography is employed to monitor head space concentrations of natural products in potato storage vessels.

H. Bioassay Methods Employed:

Potato sprout inhibition

Delivery Systems/Application Technology

* Indicates that the information is also relevant to other research areas and has been included as such.

*

Name: David H. Akey

Laboratory: Western Cotton Research Laboratory

Address: 4135 E. Broadway
Phoenix, AZ 85040

CRIS #: 5344-22-620-005-00D

Telephone: (602) 379-3524

FAX: (602) 379-4509

A. Research Accomplishments (up to 5) in Last Five Years:

1. Successfully incorporated "BT" in IPM System for production of cotton; likewise incorporated soaps and products such as M-PEDE.

2. Successfully used *Beauveria bassiana* as Naturalis L for whitefly control in cotton in the arid southwest of USA.

3. Conducted trials with the insect growth regulator, repellent, antifeedant: NEEM-Azadirachtin (2 companies products) and with numbered compounds of hydrated forms.

B. Research Objectives for Next Five years (brief description; also fill in attached table using brief descriptors):

1. Purpose: Development of information on impact of cultural conventional, and biorational chemical control approaches on pests, natural enemies, role in IPM, and resistance management. Develop "nonchemical" (conventional) alternative control methodology including behavioral chemicals, microbials, and parasites.

2. Significance:
Provide IPM tools, demonstrate use, manage insecticide resistance, and reduce use of convention "hard" pesticides.

3. Constraints (e.g., regulatory, patent, fiscal, technical, disciplinary, commercialization, etc.):
   a. Efficacy of "biorational" products
   b. Long lag time with EPA registration.
   c. Reluctance of PCA'S, consultants, and producers to achieve knowledge level high enough to really use IPM.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Industry: both small and large companies - develop new products and test them via CRADAS and other agreements. ARS & Universities promote coordinated plans for testing and incorporation of new products in control strategies.

D. Potential Uses of Research Findings:

Produce food and fiber with less adverse environmental consequences and do it more effectively.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Time frames for most products in current test for EPA registration to be achieved is 2-4 years. Opportunities exist for small business to develop "niche" markets for these products. Large companies can develop a line of "environmentally safer" products along with their conventional pesticides. See Table 1 for timeframes.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Issue of proper bioassays for growth regulator agents must be addressed.

G. Chemical Methods Employed: n/a

H. Bioassay Methods Employed:

Bioassays in green houses are conducted by treating seedling plants in a prescribed fraction of an acre or hectare. Dosages are in AI/Unit area. Other assays are performed by leaf dips into solutions of AI as parts per million. Arthropods (life stage will depend on the product under test) are then exposed to the treated plants. In some cases, the test product may be presented from a source to test fuming action at a specified distance away. Efficacy tests are conducted in field trials with all life stages; the most important efficacy criterion being percent reduction from control.

*

Name: Kenneth R. Beerwinkle, Agricultural Engineer

Laboratory: Crop Insect Pests Management Research Unit

Address: USDA, ARS, SPA, SCRL
Crop Insect Pests Management Research Unit
Rt. 5, Box 808
College Station, TX 77840

CRIS #: 6202-22000-004-00D

Telephone No.: (409)-260-9351

Fax #: (409)-260-9386

A. Research Accomplishments in Last Five Years:

1. Developed a large helicopter-towed net (5-m^2 inlet) for sampling airborne insects.

2. Characterized seasonal radar and meteorological observations associated with nocturnal insect flight at altitudes up to 900 m AGL in East-Central Texas.

3. Developed an automated, vertical-looking x-band radar system for continually monitoring aerial insect activity.

4. Characterized adult corn earworm emergence in a field corn habitat and described their feeding behavior on dallisgrass ergot honeydew.

5. Developed and olfactometer bioassay system for evaluating the efficacy of various plant volatiles as feeding attractants for adult noctuids.

B. Research Objectives for Next Five Years:

Objectives are to identify volatile chemicals of plant origin that are effective feeding attractants for adult corn earworms and other noctuids and develop synthetic mimics of those chemicals.

1. Purpose: Synthesized mimics of identified plant volatile feeding attractants will be used in formulations of toxic food baits (attracticides) for adult corn earworms and other noctuids.

2. Significance: The use of attracticide baits incorporated into pest management schemes designed to control adult pests

at their source of origin has potential for reducing crop insect pest problems with substantially reduced use of synthetic pesticides.

3. Constraints: Specific compositions of natural plant chemical compounds which cause attractancy are complex and difficult to identify.

C. Current and Future Cooperators:

ARS: P. D. Lingren, T. N. Shaver, J. D. Lopez, Jr. (College Station, TX)

D. Potential Uses of Research Findings:

With recent advancements in our understanding of aerial transport mechanisms and moth migration behaviors, coupled with the use of natural pollen analyses to identify migrants, we are rapidly approaching a capability for identifying major source areas for several species of highly-mobile crop insect pests. Successful development and implementation attracticide technologies at the source of origin for these pests has great potential for suppressing their populations over wide areas.

E. Technology Transfer and End-Use Strategies and Opportunities:

Technology transfer will be accomplished through publication of technical manuscripts and use of CRADA's and other strategies as progress warrants.

F. Thoughts on Research Needs:
G. Chemical Methods Employed:

Described by cooperator - T. N. Shaver

H. Bioassay Methods Employed:

1. Laboratory bioassays conducted with dual and multiple (up to six) choice olfactometer apparatus which operate on the Y-junction principle and were developed specifically for quantifying the responses of noctuid adults to chemical volatiles from a variety of sources.
2. Laboratory bioassays with low-speed wind tunnels.
3. Field bioassays using feeding attractant volatile sources as baits on wire-mesh cone traps.

*

Name: John M. Bland

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-018-00D

Telephone No.: (504) 286-4279

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. First Synthesis of Iturin.

2. New synthesis of B-amino acids/synthesis of iturinic acid.

3. Synthesis of Iturin analogs for SAR studies.

4. Synthesis of tentoxin analogs for SAR studies.

5. New synthesis of tentoxin.

B. Research Objectives for Next Five years (brief description):

1. Purpose: To determine the minimum structural and spatial requirements of: A. Iturin and B. Tentoxin needed for biological activity.

   Produce an economically feasible A. fungicide, B. herbicide with the environmentally-safe properties of the parent corporation.

2. Significance: Produce an economically feasible A. fungicide, B. herbicide with the environmentally-safe properties of the parent corporation.

3. Constraints: Manpower

D. Potential Uses of Research Findings:

New commercial products:
  A. Fungicide
  B. Herbicide

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

F. Thoughts on Research Needs:

G. Chemical Methods Employed:

Solution and solid phase peptide synthesis methods.

H. Bioassay Methods Employed:

Fungal inhibition zone on agar plate.
Lettuce seedling chlorosis.

Name: L.F. Bouse, J.B. Carlton, E. Franz, I.W. Kirk and M.A. Latheef

Laboratory: Aerial Application Research Unit
Southern Crops Research Laboratory

Address: 231 Scoates Hall, TAMU
College Station, TX 77843

CRIS #: 6202-22220-004-00D

Telephone No.: 409/260-936?

FAX #: 409/260-9367

A. Reasearch Accomplishments in the last 5 years:

1. Developed a data base relating spray droplet size distribution to formulation and application factors.

2. Determined the effect of aerial application variables onspray distribution on and within plant canopies, including rice, wheat, foxtail, mesquite, cotton and cantaloupe.

3. Determined effects of spray droplet size and density on the efficacy of ovicides and larvacides for control of tobacco budworm.

4. Determined the efficacy of controlled release (microcapsule suspension) formulations of pesticides against tobacco budworm on cotton.

5. Developed techniques for rapidly quantifying spray deposition on plant leaves.

B. Research Objectives for Next Five Years (brief description):

The broad objectives are to develop concepts, principles, and new technology for optimizing the efficiency and efficacy of pest control material application by aircraft.

1. Purpose: The ultimate purpose of the research is to maintain or improve control of agricultural pests while reducing the use of broad-spectrum synthetic pesticides, thus minimizing environmental hazards.

2. Significance: These objectives are applicable to a broad range of pests (insects, diseases, and weeds and brush) and their attainment would enhance the effectiveness of several control methodologies (chemicals, bacteria, virus, fungi, attracticides, pheromones, natural products, and biologicals such as parasites, predators, and nematodes).

3. Constraints: Inadequate resources and lack of linkages with microbiologists and chemists. Lack of sufficient quantities of natural products for efficacy studies using different application methodologies.

C. Current and Future Cooperators (ARS and Others):

ARS: Formulations and biological scientists.

Industry: Agricultural aircraft, spray equipment, and pesticide manufacturers.

D. Potential Uses of Research Findings:

Aerial application delivery system equipment manufacturers and commercial aerial applicators use the information obtained to improve application efficacy, efficiency and safety.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Technology is transferred through direct contact with the agricultural aviation industry, through reports of cooperative studies with private industry, and through educational programs conducted by extension specialists. Assuming the availability of natural products for application research, technology could be developed and transferred to users within five years.

F. Thoughts on Research Needs (not being addressed in other agencies or at State levels):

A major impediment to the conduct of application technology research with natural product pesticides is the lack of sufficient quantities of material for field-scale experiments. Special consideration should be given to development of scale-up techniques and systems for production of amounts sufficient for application research.

G. Chemical Methods Employed:

Evaluation of the distribution of pest control materials delivered is accomplished through use of dye tracers, water-sensitive papers, oil-sensitive papers, mylar cards, temporal samplers, and other devices.

H. Bioassay Methods Employed:

Mortality of live insects and/or plants is used as a measure of the efficacy of application treatments.

Name: Ross D. Brazee, Robert D. Fox, Charles R. Krause, Donald L. Reichard

Laboratory: Application Technology Research Unit

Address: USDA-ARS-M
Agric Engr Bldg, OARDC
Wooster, OH 44691

CRIS #: 3607-21620-003-00D, 3607-21000-002-00D

Telephone No.: 216/263-3871

FAX # 216-263-3670

A. Research Accomplishments (up to 5) in Last Five Years:

1. Assessed amount and nature of drift from spraying orchards having different tree cultivars, with different sprayer type, and under varying wind and climatic conditions.

2. Used flow simulation software to compute effect of droplet size, wind, RH, release height, and turbulence on drift distance of spray droplets.

3. Determined effect of adjuvants on spray characteristics, which required development of methods for measuring dynamic surface tension and high shear rate viscosity.

4. Developed soil injection sprayer.

5. Determined air carrier requirements for electrostatic sprayer; measured canopy penetration of spray.

B. Research Objectives for Next Five Years (brief description)

Measure and model spray drift from orchards; quantify parameters affecting in-line mixing of spray materials; characterize deposit formation using EBA; Measure interaction between spray mixture physical parameters and spray characteristics; assess plant stress factors in application; evaluate formulations and methods for biopesticide application.

1. Purpose: Identify and develop methods for applying sprayed materials with greater uniformity and with less loss to the environment.

2. Significance: Increased regulation by state, local and federal agency will restrict use of current spraying practices. New methods for applying pest control materials that are more effective must be found.

3. Constraints: Concerns about contamination of the ground water, food safety, and trespassing of sprayed materials will increase regulation of applying pest control agents. Achieving uniform coverage of plant canopies, or applying spray to specific plant sites is very difficult to achieve.

C. Current and Future Cooperators and Their Contributions (ARS and Others):

University: M. J. Bukovac (Michigan State U.), F. R. Hall (OARDC/Ohio State U.), H.E. Ozkan (Ohio State U.), Bruce Roberts (Ohio Wesleyan U.), Darrell Reneker (U. of Akron), M. Salyani (U. of Florida)

Industry: K. Reeves, Delavan Corporation; S. Pearson, Spraying Systems

D. Potential Uses of Research Findings:

Potential users will be chemical and biocontrol agent manufacturers who formulate products that are more effective and easier to spray or apply, sprayer manufacturers who can incorporate designs to improve canopy penetration or in-line mixing to reduce pesticide residue, and by applicators, both commercial and grower who can adapt spray techniques to improve application efficiency.

E. Technology and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Chemical, biopesticide and equipment manufacturers understand the need for more effective application with reduced ground water and airborne contamination. If improved formulations (from application and control point of view) and improved equipment and procedures are developed, they will probably adopt them rather quickly. Growers do not buy expensive orchard sprayers often, so even if new sprayers were available (unless forced by regulation) 10-15 years would be required for a large percentage of growers to be using new technology.

F. Thoughts on Research Needs (not being addressed in other agencies or at State Level):

I think chemical manufacturers could do a lot to improve application and effectiveness of pesticides if formulations were directed toward those goals instead of being based mainly on product stability (shelf life) and cost of inerts in formulation. Biocontrol agents will present new problems for formulations, application systems and applicators.

G. Chemical Methodologies in Use: None

H. Bioassays in Use: None

*

Name: Laurence D. Chandler

Laboratory: USDA-ARS-IBPMRL

Address: P.O. Box 748
Tifton, GA 31793

CRIS #: 6602-22000-021-00D

Telephone No.: 912/387-2326

FAX #: 912/387-2321

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identified new insect growth regulators active against corn earworm, beet armyworm and fall armyworm. These new insect growth regulators are neural agonists which are currently being developed by Rohm & Haas Co.

2. Developed a system for application of *Heliothis* NPV using chemigation technology in corn. Application of NPV significantly reduced corn earworm numbers coming from spring planted corn. Reduction of corn earworm in corn reduced the number of moths able to infest cotton.

3. Determined that treatment of corn earworm larvae with sublethal amounts of diflubenzuron and RH-5992 results in moth sterility. This information could be important in the development of area wide management programs.

4. Co-developed the product Naturalis (*Beauveria bassiana* mixed with a feeding stimulant and carrier) that is active against boll weevil, cotton fleahopper and sweetpotato whitefly.

5. Evaluated Margosan-O for use against lepidopterous pests of corn, peanut and cotton. The product has significant levels of activity against fall armyworm and corn earworm larvae when applied as either a contact material or ingested by the insect.

B. Research Objectives for Next Five Years (brief description):

Evaluation of insect growth regulators for management of lepidopterous pests of agronomic crops.

1. Purpose: To expand use of IGRs in field situations and identify methods to integrate their use with other control

measures for implementation into area wide management programs.

2. Significance: IGRs are generally non-toxic to beneficial insects and fit nicely into integrated control programs. Use of IGRs at sublethal levels and in bait formulations may provide new and innovative methods for control of certain insects over large areas. Additionally, they may be able to be combined with some naturally occurring toxicants to improve overall control of the target pest.

3. Constraints: IGRs can pose problems in application. New ways of applying low doses directly to the target organism are needed. Some regulatory problems may exist due to environmental concerns. Need studies targeted at developing combination formulations (i.e. IGR + feeding stimulant + toxicant such as neem or other natural product or biological).

Development of area wide management of corn earworm using NPV applications on corn (In cooperation with J. Hamm).

1. Purpose: To expand NPV applications over larger corn acreage throughout the southern states. Comparison of application methods will be made to determine optimal way of getting the virus to the target.

2. Significance: NPV is a naturally occurring virus that can be easily manipulated for mass application. Development of management strategies for control of corn earworm using NPV in combination with other biological and natural control agents would improve the likelihood for this method to be developed into a large scale management program.

3. Constraints: NPV commercial production is limited. An effort is needed to increase interest in this material in the private sector. Additionally, any program targeting field corn will probably needed to be subsidized by a government agency in order to be implemented. Corn economics will not allow growers to pay for the entire cost of a program of this type.

Evaluation of new natural products for use in lepidopterous insect management programs in agronomic crops.

1. Purpose: To identify new natural products (commercially available or from government/University labs) that might be useful in control of beet armyworm, fall armyworm, corn earworm and tobacco budworm.

2. Significance: Any addition to our current knowledge of

natural product activity against the named pests will improve our ability to manage the pests. Materials such as limonoids, neem oil and derivatives, and capsaicin are currently being evaluated in our lab and may prove important in future insect control efforts.

3. Constraints: Many natural compounds are not available in large amounts needed for field tests. Additionally, they are very expensive to obtain.

C. Current and Future Cooperators (ARS and Others):

John J. Hamm, USDA-ARS, Tifton, GA - NPV and Bt evaluation
Jim Carpenter, USDA-ARS, Tifton, GA - IGR sterility effects
Harold Sumner, USDA-ARS, Tifton, GA - application technology
Mickey McGuire, USDA-ARS, Peoria, IL - IGR formulations
Baruch Shasha, USDA-ARS, Peoria, IL - IGR formulations
James Wright, USDA-ARS, Weslaco, TX - Naturalis cooperator
Gary Herzog, Univ. GA, Tifton, GA - field evaluations
Edd Harrison, Rohm & Haas Co., Camilla, GA - IGR applications

D. Potential Uses of Research Findings:

The studies outlined here will be useful for growers and the private sector insecticde manufacturer. These studies should also be important in development of area wide management programs for targeted pests (an ARS priority).

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Information will be transferred for growers, other scientists, action agencies and private industry as research results become available. Private industry should have interest in development of new products or combinations of products that are environmentally friendly. Action agency opportunities are dependent upon development of area wide management strategies that may take up five years to develop.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Need studies designed at integrating several control strategies, including natural products/biologicals, into an effective management program. Need additional help from chemists-formulation experts on development of formulations that will remain active for longer periods of time. Need nationwide coordination of programs using the best available biologically friendly control tactics to avoid unnecessary

duplication of studies.

G. Chemical Methods employed:

N/A

H. Bioassays in Use:

Bioassays used consist of topical, leaf dip, diet spread, and diet incorporated methods. In addition a spray table is used to simulate actual field applications. Treated leaves are removed from treated plants and held in cups to evaluate insect mortality.

*

Name: William J. Connick, Jr. and Donald J. Daigle

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-22000-001-00D

Telephone No.: (504) 286-4527

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Established germination rate as a criteria for the successful formulation of *Alternaria cassiae*, a mycoherbicide for the weed, sicklepod (*Cassia obtusifolia L.*).

2. Developed improved invert emulsion formulations for mycoherbicides, reducing dew requirements.

3. Developed a new formulation concept using wheat flour to make granules called "PESTA" containing biocontrol fungi and nematodes.

B. Research Objectives for Next Five years (brief description):

To discover and develop new or improved formulations for biocontrol agents.

1. Purpose: Some primary goals are to control the weed, hemp sesbania, in fields, to improve formulation technology with the use of a mycoherbicide with a cheap, practical delivery system, and to develop an effective granular formulation containing entomopathogenic nematodes. Methods to increase shelf life of the biocontrol agents are being investigated.

2 Significance: Lack of suitable formulations is a major constraint to further acceptance of biopesticides. Biocontrol products can reduce the amount of pesticide applied to crops such as soybeans and thus reduce the farmers cost and/or reduce the effect of pesticides on the environment. Biopesticide products with adequate shelf life will help the farmer and homeowner.

3. Constraints: Products with adequate shelf life and efficacy are scarce. Only three mycoherbicide products have been commercialized in the last 15 years.

Use of insect-killing nematodes is a promising application, but improved formulations are needed. Granular products would fill an important niche. More formulation research by industry, universities, and ARS is needed.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Numerous ARS scientist engaged in biocontrol research. Among them are Dr. Doug Boyette, Stoneville, MS, USDA, ARS; and Dr. Mark Jackson, Peoria, IL, USDA, ARS. Dr. P.C. Quimby, Jr., Bozeman, MT and Dr. Bill Nickle, BARC-W.

D. Potential Uses of Research Findings:

To broaden the base of cooperative research and to bring biocontrol formulations closer to commercial acceptance.

E. Technology Transfer and end Use Strategies and Opportunities:

Two companies have applied for exclusive rights to Pesta containing insect-killing nematodes. With suitable effort, a biocontrol product could be marketed within 3 years.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Strengthen the research on formulation and application technology.

G. Chemical Methodologies in Use:

Numerous chemical pesticide formulations are available, but the technology used does not usually apply to delicate, living biocontrol agents.

H. Bioassays in Use:

Bioassays are usually performed by the biological scientists we cooperate with. However, we sometimes evaluate weed control on seedlings in the greenhouse.

Name: Oliver D. Dailey, Richard M. Johnson, and Armand B. Pepperman, Jr.

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-057-00D

Telephone No.: (504) 286-4527

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Developed an alginate granular formulation for the controlled release of the herbicides atrazine, alachlor and metribuzin. Extended release with reasonable efficacy was demonstrated for all herbicide formulations. This formulation is based on alginate-kaolin-linseed oil.

2. Soil column experiments were performed to determine the mobility of herbicides from CRF's. All CRF;s reduced herbicide mobility to some extent, those formulations that contained linseed oil uniformly and significantly decreased herbicide mobility.

3. Polymeric microcapsules of these same herbicides were prepared and evaluated. Microcapsules of all three herbicides prepared with ethyl cellulose and cellulose acetate butyrate showed the most promise. The atrazine formulations exhibited extended controlled-release properties and herbicidal activity superior to the commercial formulation.

B. Research Objectives for Next Five years (brief description):

Experiments will continue to investigate the environmental fate of CRF's compared to technical and commercial formulations. Those areas to be investigated include: herbicide mobility, persistence, volatility and runoff potential.

1. Purpose: To develop pesticide formulations that minimize environmental contamination while maintaining efficacy. To decrease air, surface water, and groundwater contamination by the development of controlled release formulations of pesticides.

2. Significance: Controlled release formulations (CRF) of herbicides have the potential to reduce environmental contamination while still providing effective control. In

addition, since the CRF protects the herbicide from losses to volatilization, leaching, photodegradation, etc., reduced application rates should be possible.

3. Constraints: Maintaining efficacy at the required level and minimizing environmental contamination.

C. Current and Future Cooperators and their Contributions (ARS and Others):

1. Dr. Clyde C. Dowler, Research Agronomist, USDA, ARS.
2. Dr. Mohamed A. Latheef, Research Entomologist, USDA, ARS.
3. Dr. H. M. Selim, Agronomy Department, LSU.
4. Dr. Sammie Smith, USDA, ARS.

D. Potential Uses of Research Findings:

Development of controlled-release formulations of selected herbicides and insecticides which are safer to handle and minimize environmental contamination while maintaining efficacy. CR herbicide formulations have the potential to replace current herbicide formulations in many markets. The biggest potential may be in the high-value markets such as: cash crop producers, horticultural producers, commercial greenhouses and turf/lawn care markets.

E. Technology Transfer and End Use Strategies and Opportunities:

The intent is to produce base-line data for the choice of the proper formulation for minimal environmental contamination with acceptable efficacy. Some of the products may have application to special niches such as nursery and ornamentals, turf and rangeland.

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Determination of optimal level of application for effective control with minimal environmental contamination.

G. Chemical Methodologies in Use:

Presently using HPLC, TLC, radiation assay, GC/MS and will be using immunoassay soon.

H. Bioassays in Use:

We are dependent upon cooperators for bioassays as we have no biological people on board. Assays are generally whole plant in greenhouse pottings.

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Name: Paul Engel

Laboratory: USDA, ARS, SRRC, ET Research Unit

Address: P.O. Box 19687
New Orleans, LA 70179

CRIS #: 6435-41000-018-00D

Telephone No.: (504) 286-4375

FAX #: (504) 286-4367

A. Research Accomplishments (up to 5) in Last Five Years:

1. Tagged nikkomycin (a fungicide produced by *Streptomyces tendae*) genes with transposon Tn4560, cloned DNA flanking Tn4560 insertion site and used this flanking DNA to do gene disruption to verify that flanking DNA indeed specifies nikkomycin genes.

B. Research Objectives for Next Five years (brief description):

Clone entire gene cluster of genes required for nikkomycin biosynthesis.

1. Purpose: To isolate genes so they can be moved into plants - self protection against fungal diseases.

2 Significance: Nikkomycin is a safe (chitin synthase inhibitor) fungicide that could protect food-stuff from decay.

3. Constraints: Management does not support science at present time.

C. Current and Future Cooperators and their Contributions (ARS and Others):

Dr. David Kingston, Department of Chemistry, Virginia Tech; doing MS and NMR to determine structure of substance accumulated by a nikkomycin mutant.

D. Potential Uses of Research Findings:

Safe and effective fungicide to increase shelf-life of foods and as self-protectant against fungal diseases in transgenic plants.

E. Technology Transfer and End-Use Strategies:

F. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Sufficient technical assistance is needed.

G. Chemical Methods Employed:

H. Bioassay Methods Employed:

*

Name: Kevin D. Howard

Laboratory: Application Technology Research Unit

Address: Stoneville, MS 38776

CRIS #: 6402-21220-003-00D

Telephone No: 601-686-5240

Fax: 601-686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. Designed and developed mechanical shields for conventional hydraulic sprayer which will rotate the crop canopy exposing the bottom side of the leaves for treatment of insecticide.

2. Evaluated air-assisted sprayers for driftcontrol,penetration and deposition on both upper and lower leaf surfaces, and control of both weeds and insects.

3. Conducted drift studies with dual systems and dual traces to accurately evaluate different spraying technology for drift control and to evaluate traces, both fluorescent dye and chemical tracers to determine the simplest to used and detect.

4. Investigated the transient time and the efficiency of concentration mixing both from nozzle to nozzle long the spray boom and as a function of time for a direct in-line injection sprayer at three injection points, low pressure side of the system pump, high pressure side of the system pump, and directly at the nozzle.

B. Research Objectives for Next Five Years (brief description):
Off Target Drift from Aerial Application

1. Purpose: To determine the parameters which influence off target drift from aerial application of agricultural pesticides.

2. Significance: There are several parameters of aircraft design, nozzle design and orientation, and environmental parameters which influence off target drift of agricultural sprays. These parameters need to be established in order to eliminate off target drift while increasing spray deposition and penetration.

3. Constraints: Aircraft and support facilities are needed, as well as an easily detectable tracer which is stable in sun light and nontoxic to humans and a reliable method for measuring drift.

Off Target Drift from Agricultural Ground Sprayers

1. Purpose: To determine the parameters which influence off target drift of ground application equipment.

2. Significance: There are several design parameters of a agricultural ground sprayer, nozzle design, nozzle configuration, and the use of air-assistance which will influence off target drift of agricultural sprays. These parameters need to be established in order to eliminate off target drift while increasing spray deposition and penetration.

3. Constraints: An easily detectable tracer which is stable in sun light and nontoxic to humans and a reliable method for measuring drift.

Direct In-line Injection of Agricultural Chemicals

1. Purpose: To develop a spraying system that will inject the undiluted chemical, at the desired rate, into the diluent flow at a point just prior to atomization of the diluent.

2. Significance: The capability of injecting the undiluted chemical into the diluent flow would aid in the elimination of calibration errors and the need for excess chemical disposal.

3. Constraints: Even distribution of the chemical between nozzles and even mixing of the chemical within the nozzle.

Closed Containment System for Agricultural Chemical

1. Purpose: To develop a system allowing the transfer of the chemical from the manufacture's container to the chemical holding tank on the sprayer.

2. Significance: With increased concern of pesticide applicator exposure and with new worker and handler regulations, a method of being able to transfer the undiluted chemical from one tank to the next would reduce handler exposure.

3. Constraints: Different size containers and container openings, and being able to handle all of the different pesticide formulations.
Pesticide Activity

1. Purpose: To determine the effect droplet size, adjuvant, method of application, ground versus aerial application, and application rate of both pesticide and carrier volume has on pesticide activity.

2. Significance: This work would aid in determining the best application method which would result in the greatest pesticide activity.

3. Constraints: Facilities which can handle the testing with pesticides and disposal of the pesticide wast.

C. Current and Future Cooperators ARS and Others:

ARS: Joseph E. Mulrooney, William Scott, and Charles Bryson (Stoneville, MS)

Industry: Larry Gaultney E. I. DuPont

D. Potential Uses of Research Findings:

Research findings from the aerial and ground drift tests will aid in the design and configuration of the application system to reduce off target drift and increase pesticide placement and activity. Findings from the direct injection/closed container system would eliminate over and under application of pesticides and the need for disposal of excess tank mixes. This system will also reduce applicator exposure due loading and mixing of pesticides, and the deposal of pesticide containers. The determination of the most appropriate application method for applying a given pesticide will also aid in the reduction of pesticide wast due to increased pesticide activity.

E. Technology Transfer and End-Use Strategies and Opportunities (include Predictive Timeframes):

Transfer of technology through publications, coopertive research agreements, interpersonal interactions, meetings, and workshops. The end-use strategies and opportunities will be to take the final product to the grower, wheather through a manufacturer or the extension service. The time frame for the information transfer will be as soon as there is something that will be beneficial to the grower.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

ARS has very limited personnel available to conduct research in the area of application technology. There are many new and exciting methods of applying agrochemicals and manufactures are making claims that my not always hold true

to form. We need to investigate these new methods of application and determine which concept is the most appropriate to accomplish the needs of the consumer.

G. Chemical Methods Employed:

Pesticide residue analyses by gas and liquid chromatography, and analyses of fluorescent dye by fluorometer.

H. Bioassay Methods Employed:

Insecticide bioassays in the laboratory and field, and herbicide bioassays in the field.

*

Name: J. Allen Miller

Laboratory: Knipling-Bushland U. S.
Livestock Insects Research

Address: 2700 Fredericksburg Road
Kerrville, TX 7802

CRIS #: 6205-32000-007-00D
6205-32000-009-00D
6205-32000-010-00D
6205-32000-011-00D

Telephone No.: (210) 257-3566
(210) 792-0321

Fax No.: (210) 792-0314

A. Research Accomplishments (up to 5) in Last Five Years:

1. Determined efficacy, pharmacokinetics, and optimal dosage for avermectins (ivermectin, moxidectin, doramectin) for livestock pests.

2. Developed delivery systems for oral and subcutaneous treatments with ivermectin.

3. Demonstrated efficacy of neem (azadirachtin) against larval horn flies in manure.

4. Determined efficacy and optimal dose of selected IGRs against biting flies.

5. Developed medicated-bait system for control of ticks feeding on cattle and deer.

B. Research Objectives for Next Five Years (brief description):

The broad objective is the development of delivery systems to improve the effectiveness, efficiency and safety of livestock pests control.

1. Purpose: to explore and develop natural products for the control of livestock pests; to determine the efficacy and means of application of 2nd generation avermectins; to develop microbials of horn fly control; to develop delivery systems to enhance the effectiveness and safety of these agents.

2. Significance: Although the use of synthetic pesticides in livestock pest control constitutes a minor use, it is nevertheless desirable to reduce the quantity and dependence on such materials. The availability of environmentally friendlier control agents will improve the safety to livestock, applicators, and the consumer. Natural control agents will broaden the arsenal of control tools.

3. Constraints: The largest constraint is the unavailability of effective natural control agents. The discovery of such materials is a weak link. The registration process and the commercialization of minor use products for livestock pests control are also constraints. Establishment of priority and commitment by ARS to the work is needed.

C. Current and Future Cooperators (ARS and Others):

We will maintain, cultivate and initiate cooperation with other ARS Laboratories, Universities, and Industry known to be working on discovery and development of natural products for use in control of plant pests in order to explore their potential against livestock pests.

Industry cooperators include Merck & Co., American Cyanamid, Pfizer, and others.

D. Potential Uses of Research Findings:

The development of new control agents and delivery systems will increase our arsenal of tools available for control of livestock pests. It will reduce the use of synthetic pesticides by enabling more efficient delivery to the target pest in minimal quantities necessary for control. The research should lead to safer control agents both in terms of environmental and consumer concerns.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Current patents are licensed to private industry and being commercialized. Where appropriate, patents will be sought for new technology. In other cases, technology will be transferred through publication and dissemination of findings. CRADAs will be established to expedite research and gain support from private industry. Past experience has shown us that time frames for such activities are very unpredictable. Current research on injectable microspheres for tick control has important applications in the fever tick quarantine areas of South Texas. Work on the medicated-baits for control of ticks on deer should find application in Lyme Disease control.

F. Thoughts on Research Needs (not being addressed in other Agencies or at the State levels):

The weak link in this process is that of discovery of natural products for livestock pests control. It seems unlikely (because of the minor use status for livestock pests control) that industry will invest significantly in the discovery process. A word of caution: Because many of these natural products may be as toxic (or more toxic) as synthetic pesticides, a system must be developed equivalent to the MSDS information to enable movement of these materials from laboratory to laboratory in a safe manner. ARS should develop a protocol for such a system.

G. Chemical Methods Employed:

HPLC is used for determination of serum levels of drug resulting from experimental delivery systems .

H. Bioassy Methods Employed:

Standard bioassays have been developed for efficacy of agents against biting flies and ticks.

Name: Joseph E. Mulrooney *

Laboratory: Application Technology Research

Address: P. O. Box 350
Stoneville, MS 38776

CRIS#: 6402-21220-003-00D

Telephone No.: 601-686-5342

FAX#: 601-686-5422

A. Research Accomplishments (up to 5) in Last Five Years:

1. Clarified the effects of cotton allelochemicals (gossypol, tannin, and anthocyanin) on tobacco budworm feeding and growth.

2. Determined the effect of PI, a plant growth regulator, on the allelochemical content of cotton and subsequent effects on the growth of tobacco budworm larvae.

3. Determined the effect of tobacco budworm infestation on the fruit distribution of cotton varieties infested at different developmental stages.

4. Demonstrated differences in growth rates of tobacco budworm larvae from lab populations differing in the amount of time in culture since outcrossing to the wild.

5. Evaluated the deposition and efficacy of insecticides applied by air and ground spray equipment on control of tobacco budworm and soybean looper in cotton and soybeans.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop new and innovative insecticide application methods to improve control of insect pests.

1. Purpose: To decrease insecticide application rates and the amount of insecticide moving off-target.

2. Significance: The costs for insect control to the producer and to the environment are increasing. Application methods to decrease these costs are a high priority.

3. Constraints: Aircraft and support facilities.

C. Current and Future Cooperators ARS and Others:

ARS: Kevin Howard, Lavone Lambert, and William Scott (Stoneville).

Industry: Marcus Adair and Gary Melchior (Abbott Labs).

D. Potential Uses of Research Findings:

The relationship between drop size, off-target movement, and efficacy would benefit aerial applicators and consultants. Elucidation of the mechanisms of insecticide transfer from the plant surface to the insect would aid in the development of insecticide formulations that are persistent on the plant and readily transferred to target insects. Determining the effect of aircraft design and spray-boom and nozzle type on the efficacy of insecticides would be of benefit to the chemical and application industries.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Transfer of technology through publications, cooperative research agreements, interpersonal interactions, workshops, and meetings.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels:

Personnel and resource expenditures for application research are very limited even though improving pesticide application is a high priority of the application and chemical industries. While the discovery of safer and more environmentally friendly pesticides is important, improving the application of those that are now on the market deserves equal attention.

G. Chemical Methods Employed:

Insecticide residue analyses by gas and liquid chromatography.

H. Bioassay Methods Employed:

Insecticide bioassays in the laboratory and field.

Name: William C. Nettles, Jr.

Laboratory: Subtropical Agricultural Research

Address: 2413 East Highway 83
Weslaco, TX 78596

CRIS #: 6204-22000-006-00D
6204-22000-006-01T

Telephone No.: 210-969-4868

FAX #: 210-969-4888

A. Research Accomplishments (up to 5) in Last Five Years:

1. Confidential because of pending patents and CRADA funded by CIBA-Geigy. Results are centered around development of artificial diets and identification of growth factors and ovipositional stimulants from host insects.

B. Research Objectives for Next Five years (brief description):

1. Purpose: Mass production of parasitic insects for control of pest insects, specifically identify ovipositional stimulants, growth factors, and possibly antibiotics.

2. Significance: Provide more effective control of pest insects without the use of chemical insecticides.

3. Constraints: Technical, fiscal.

C. Current and Future Cooperators (ARS and Otherwise):

CIBA-Geigy: A total of almost $400,000 over the last 5 years.

Several other companies likely would be involved except for CIBA-Geigy support.

D. Potential Uses of Research findings:

Large scale automated in vitro production of *Trichogramma* spp. (egg parasites) and tachinids (parasitic flies that attack pest larvae such as *Heliothis*, *Helicoverpa*, gypsy moth, etc.) for field release and control of pest insects.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

CIBA-Geigy has the right of first refusal and likely will

purchase licenses to use the several patents that will be obtained. Time: 2-5 years.

F. Thoughts on Research Needs (not being addressed in other agencies or at State levels):

Much greater chemical and engineering expertise is needed. Work needs to be located in a less isolated area where there are better opportunities for greatly expanded interactions with scientists from a broad range of disciplines (analytical chemistry, biochemistry, agricultural, mechanical and chemical engineering, microbiology).

G. Chemicals Methods Employed:

Liquid and HPL

H. Bioassay Methods Employed:

Oviposition into artificial eggs. Growth and development of purified extracts added to artificial diets.

*

Name: David C. Robacker

Laboratory: Subtropical Agricultural Research

Address: 301 S. International Blvd.
Weslaco, TX 78596

CRIS #: 6204-43000-005-00D

Telephone No.: 210/565-2647

FAX #: 210/565-6652

A. Research Accomplishments (up to 5) in Last Five Years:

1. A chemically defined protein-type attractant for both male and female was developed.

2. A chemically defined attractant for both males and females was developed from host fruit volatiles.

3. Attractiveness of pheromone to both males and females in the field was demonstrated.

4. It was shown that bacteria of numerous species from at Least five families are generally attractive to Mexican fruit fly.

5. Differential attractiveness of host-fruit and protein-type baits was linked to adult nutrition and physiology.

B. Research Objectives for Next Five Years (Brief Description):

The objectives are to develop an attractant for a dry trap that is superior to currently used baits and to understand how it works.

1. Purpose: The purpose of the work is to replace the standard McPhail trap containing protein bait currently used to monitor populations of *Anastrepha* fruit flies with a new, more effective dry trap with a chemically defined bait that can emit attractive amounts of chemicals for at least twice as long as currently used traps.

2. Significance: Such a dry trap should be less expensive than the McPhail trap both in actual cost of traps and in manpower needed to run trapping programs. More importantly, more effective traps translate into earlier detection of fly populations and therefore faster and less expensive

eradication.

3. Constraints: Current slow-release technology is inadequate. I also need more analytical chemistry support for identification of bacteria-produced attractants and cooperators for field testing of new traps with the various *Anastrepha* species.

C. Current and Future Cooperators (ARS and Others):

ARS: R. Flath (Albany, CA); R. Heath (Gainesville, FL); E. Jang (Hilo, HI)

APHIS: A. Martinez, K. Esau (Mission, TX); D. Chambers (Guatemala City, GUA)

Industry: W. Denton (AgriSense); J. Jenkins (Scentry)

D. Potential Uses of Research Findings:

Discussed above was replacement of the expensive McPhail trap/protein bait trapping system with less expensive dry traps containing long-lasting chemically defined attractants. In addition, this research will increase our understanding of complex relationships of environmental and physiological factors affecting attraction of fruit flies to various baits. Such knowledge could enable users to make informed choices regarding which baits to use under a specific set of conditions and could aid researchers working with other species of Tephritidae to better understand their systems. Finally, this research may introduce new chemical classes as attractants that may function in novel ways.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

Development of slow-release technology for these types of chemicals will be conducted by industry during the next year. The attractant technology will be transferred to my industry partner for commercial production by 1995. Large scale pilot testing perhaps in California with CDFA or in Texas with TDA is anticipated for 1996-97.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

N/A

G. Chemical Methods Employed:

Methods used in my laboratory include various volatile collection techniques, on-column and Grob injection capillary GC, and HPLC.

H. Bioassay Methods Employed:

A rapid laboratory bioassay called a "cage-top" bioassay is used for initial screening of most chemicals. A flight chamber type wind tunnel and local field testing with sterile, released Mexican fruit flies are used to evaluate chemicals and mixtures that seem promising. Field testing in Mexico and Guatemala is employed to evaluate attractants deemed best in local field testing.

Name: Baruch S. Shasha, Research Chemist
Michael R.McGuire Res. Ent./Lead Scientist

Laboratory: Plant Polymer Research

Address: USDA-ARS-NCAUR
1815 N. University St
Peoria, Il 61604

CRIS #: 3620-41000-045-00D

Telephone No.: 309-681-6222

FAX: 309-681-6651

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovery and evaluation of a new granular formulation for microbial pesticides based on pregelatinized starch. Granule remains discrete through wet and dry periods.

2. Discovery and evaluation of an additional granular formulation capable of adhering to surfaces.

3. Discovery and evaluation of a sprayable formulation for microbial pesticides that resists wash-off by rainfall.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: Our overall objective is to improve efficacy and field persistence of pest control agents through new formulations.

2. Significance: Currently, microbial pesticides are used sparingly. New formulations should enable more wide spread and efficacious use of these biological agents.

3. Constraints: Our largest constraint is in getting industry to accept and test the new formulations even though we have several years of efficacy data suggesting starch formulations do add to field persistence and efficacy of microbial insecticides. Technology transfer should be more highly emphasized with the addition of staff to act as liaisons between industry and the bench.

C: Current and Future Cooperators (ARS and Others):

ARS: R. Bartelt, K. Eskins (NCAUR); J. Lewis, S. Meyers, B. Nickle (BARC); L. Chandler (Tifton, GA); D. Streett (Bozman, MT).

D: Potential Uses of Research Findings:

If successful formulations are developed that extend or enhance microbial insecticides, potentially, microbials may replace some of the chemicals currently being used. Additionally, new markets for starch and flour (currently in surplus) will be created. Although most of our work has been done with microbials, the formulations are versatile enough to allow formulation of virtually any component.

E: Technology Transfer and End-Use Strategies and Opportunities (including predictive Time Frames):

Generally, our technology is transferred through direct communication with private industry. Currently, we are working with the Biotechnology Research and Development Company (a consortium of several companies) to transfer our technology. They have sublicensed our technology to 4 companies and have obtained funding through AARC to scale-up our processes.

F: Thoughts on Research Needs (not being addressed in other Agencies or at State Levels):

Research on biological control is scattered among various locations. Prioritizing research needs with concomitant levels of funding is necessary from an administrative level.

G: Chemical Methods Employed:

Chemical Processes Employed: The starch encapsulation process is relatively simple. Pregelatinized starch or flour is mixed with water and active agents. Granules can be formed or, if low amounts or starch are added to a spray tank, a film will form after spraying. No hazardous chemicals are involved and the end product will biodegrade.

H: Bioassay Methods Employed:

Standard feeding bioassays are conducted to determine efficacy and residual activity of microbial insecticides. Insects used include *Ostrinia nubilalis*, *Heliothis virescens* and *Trichoplusia ni*. Laboratory, greenhouse and field experiments are carried out in our location to fully assess the activity of the starch formulations.

Name: Harold R. Sumner

Laboratory: Insect Biology & Population Management Lab, Agricultural Engineer

Address: P. O. Box 748
Tifton, Georgia 31793

CRIS #: 6602-2200-021-00D

Telephone No.: 912-387-2347

FAX #: 912-387-2321

A. Research Accomplishments (up to 5) in Last Five Years:

1. Designed and developed rotary applicator systems for artificially infesting host plants in large and small research plots with newly emerged fall armyworm larvae. The equipment provides a method of rapid and uniform infesting of host plants for evaluation using natural or chemical pesticides.

2. Developed a low cost center pivot irrigation attached sprayer and an irrigation system simulator for application of pesticides and bio-control materials.

3. Developed and evaluated direct injection methods for chemigation and sprayer systems to apply pest control materials.

4. Evaluated six sprayer systems for the application of biological insecticides that improve the placement of materials on the underside of cotton leaves. Sprayers included electrostatic, air assist, shielded spray nozzles and conventional hydraulic nozzles.

B. Research Objectives for Next Five Years (brief description):

1. Purpose: In cooperation with entomologist, evaluate methods for application of important natural products that will complement bio-control and chemical control methods and integrate them into present management systems. Develop chemigation, irrigation system attached sprayers, and ground sprayer systems that efficiently apply natural products.

2. Significance: Application systems that efficiently apply natural products will improve pest control, product selection, and increase the number of acceptable materials. Natural products can be substituted for chemical pest control materials, thereby reducing some

of the personal and environmental risk associated with pest management.

3. Constraints: Formulations may not be compatible with present application methods. Spray nozzles and application systems are not available for the wide range of natural products materials and types under consideration.

C. Current and Future Cooperators (ARS and Others):

Larry D. Chandler, Entomology, USDA-ARS, Tifton, GA
Gary A. Herzog, Entomology, University of Georgia, Tifton, GA
Clyde C. Dowler, Weed Science, USDA-ARS, Tifton, GA
John J. Hamm, Insect Pathology, USDA-ARS, Tifton, GA

D. Potential Uses of Research Findings:

Develop management strategies for insect and weed pest that emphasize reduced chemical pesticide inputs.

E. Technology Transfer and End-Use Strategies and Opportunities (Include Predictive Timeframes):

To transfer natural product technology to growers and producers. Develop other associated natural products by industry. Include natural products in area-wide management programs to control certain pests with minimum environmental problems.

F. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Resources and SY's limited
Need cooperation with industry
Formulations and application methods need to be developed

G. Chemical Methods Employed:

None

H. Bioassay Methods Employed:

None

Name: William T. Wilson, Ph.D.
Anita M. Collins, Ph.D.

Laboratory: USDA-ARS Honey Bee Research Unit

Address: 2413 E. Hwy. 83
Weslaco, TX 78596

CRIS # 6204-21000-004-36 (mite control)
6204-21000-005-37 (Africanized bee)

Telephone: (210)969-4870

FAX: (210) 969-4884

Research Categories: C. Natural products for parasite & pathogen control and E. Delivery systems/application technology for natural products.

A. Research Accomplishments in Past 5 Yrs.:

1. Developed and tested dosages of menthol crystals needed for fumigation and control of tracheal mites (*Acarapis woodi*) in adult honey bees (*Apis mellifera*). Now registered (EPA) for use in honey bee colonies.

2. Formic acid fumes were shown to be an effective control for the internal mite, *A. woodi*, and for the external mite, *Varroa lacobsoni*, in honey bees.

3. Field testing of menthol in a liquid carrier sprayed into the air as an aerosol to repel or discourage defensive adult Africanized honey bees from following people.

4. Field testing and employment of organic smoke and sugar syrup for reducing defensiveness in Africanized honey bees.

B. Research Objectives for Next 5 Yrs.:

The broad objective is to develop and enhance technology to improve the performance of honey bees for efficient pollination and to maximize honey production through the use of natural products.

1. <u>Control of Parasitic Mites in Honey Bees</u>. (Purpose) Continued testing of naturally occurring products for the control of parasitic mites on honey bees. (Significance) Successful mite control would result in increased honey production, better pollination and improved colony survival during winter in northern climates.

2. <u>Reducing the Level of Defensiveness in Africanized Bees</u>.

(Purpose) Further testing of natural products for repelling or calming aggressive honey bees, especially those of African ancestry. (Significance) Effective compounds would help in protecting people outdoors from possible attack by Africanized honey bees and reduce the risk of defensive bees following a beekeeper from a bee yard.

3. Constraints. Limited funds and personnel. Inadequate number of Africanized honey bee (AHB) colonies in the U.S. Inherent problems with conducting research in Mexico where many AHB colonies exist.

C. Current and Future Cooperators:

The primary research has been conducted by USDA-ARS personnel in cooperation with commercial beekeepers in the U.S. and Mexico. Informational exchange on formic acid research has taken place with personnel in the British Columbian Dept. of Agriculture in western Canada.

Texas A & M University @ Weslaco, TX: Dr. Frank Eischen.

University of Nuevo Leon @ Linares, N.L., Mexico: Dr. Celina Garza Q.

Tamaulipas Department of Agriculture: Various professional personnel including entomologists and veterinarians.

D. Potential Uses of Research Findings:

Menthol and formic acid treatments for parasitic mite control are already being used by the beekeeping industry in the U. S. and in Canada. Formic acid application does not yet have EPA approval in the U. S. but approval has been granted in Canada by the appropriate agency. The use of natural miticides will likely increase during the next 5 years. Aerosols for modifying the behavior of Africanized bees has potential value for the beekeeping industry of the U. S. and Mexico but potential use by the general public could be extensive in hot climates.

E. Technology Transfer and End-Use Strategies:

Technology transfer has already occurred with the extensive use of both menthol and formic acid by the U. S. beekeeping industry. Aerosol repellents for use in modifying Africanized bee behavior will probably be utilized and products marketed for public use within the next 5 years.

F. Thoughts on Research Needs:

Natural products appear to have a good future because they seem to be more acceptable to the general public. Some people perceive

them to be less threatening in terms of toxicity.

G. Chemical Methods Employed:

Utilized gas chromatography (GC) in the development and modification of assays for detecting residues of formic acid, menthol and amitraz. GC identification of cuticular hydrocarbons on AHB for subspecies determination.

H. Bioassay Methods Employed:

In studies with natural repellents, we measure the reduction in the number of flying bees around an experimenter (person) and the number of stings in leather targets from disturbed bee colonies. Dissection of adult bees to expose tracheal tubes for counting the number of live and dead adult *Acarapis woodi* mites.

APPENDIX D
PLENARY SESSIONS

CHALLENGES AND NEEDS FOR NATURAL PRODUCTS TO CONTROL AGRICULTURAL PESTS

Presented by
Ruxton H. Villet
Deputy Assistant Administrator
Office of Technology Transfer

Industrial entrepreneurs who dismiss green consumer trends as bothersome do so at their peril. Far better is it to embrace the inherent market opportunities. Nine advantages of natural product biopesticides are pointed out. Investor interest in ag-biotech is discussed. The international challenge is elaborated, including the need for collaborative international exchange in natural products technology. A great deal of this technology is emerging outside the U.S. and requires identification and monitoring.

Due to efforts by the Agricultural Research Service's (ARS) Office of Technology Transfer, ARS, at present, is in a somewhat commanding position in the whole Federal ensemble in regard to commercialization. About one-third of the CRADA's within ARS are on biological control. This is some guide to prevailing industrial interest. Redirection of research funds toward products of interest to the marketplace could create a useful pool of funds for natural biopesticide development. Bioprocess engineering, as a crucial path to marketable biopesticides, is discussed briefly.

The address ends with a very recent report on surprising German strategic developments in value added green technologies. This serves to confirm Dr. Hank Cutler's predictions, some years ago, about foreign dynamism in this area.

OPPORTUNITIES FOR EXTRAMURAL COOPERATION: THE RIPS-ARS CONNECTION

NATURAL PRODUCTS DEVELOPMENT CONSORTIUM

Presented by
James D. McChesney
RIPS, University of Mississippi
University of MS, 38677

For American technology to advance in the face of higher technological competitiveness from around the world, some new thinking is required. The federal government funds billions of

dollars for research in federal laboratories and agencies, at universities and non-profit institutes. The most direct way for the people of the U.S. to benefit from this investment in research is for products and services to flow from these research endeavors. Yet that is not happening most effectively. Rather, it is widely perceived that research discoveries and developments remain largely unexploited until they are picked up by international competitors, transformed into products which are then marketed globally, thus providing much of the basis for continued economic development for international competitors rather than for the United States. Can this pattern be altered? How can the economic development of the United States be fostered by the immense federal investment in research, especially by investment in research for the discovery and development of new pharmaceuticals and agrochemicals? This can only happen effectively if there is formed a partnership of entities with the complimentary strengths of discovery, development <u>and</u> commercialization. Those complementary strengths are to be found in universities, federal and state laboratories and the private sector. The Research Institute of Pharmaceutical Sciences of the University of Mississippi has formed, and is leading a partnership, of a university research organization (the Institute, RIPS), the federal government [the Agricultural Research Service, (ARS) and the Cooperative State Research Service, (CSRS) of the USDA] and pharmaceutical and agrochemical industries by establishing the **Natural Products Development Consortium** of the **National Center for the Development of Natural Products.** The **Consortium** takes advantage of the strengths of each partner to discover, develop and commercialize new pharmaceuticals and agrochemicals derived from natural products.

The potential for developing new sources of valuable plant chemicals is largely unexplored and the benefits from doing so unexploited. Plants are known sources of insecticides, herbicides, medicines, and other useful substances; developing new industries and crops based upon plant extracts and extraction residues provides opportunities for agricultural and industrial expansion that will benefit farmers, consumers and industry.

New-crop and plant-product development will,

- provide industries with alternative and renewable sources of raw materials
- diversify and increase efficiency of agricultural production
- improve land resource use
- offer increased economic stability to farmers
- create new and improve existing agriculturally related industries
- increase employment opportunities
- provide industries with alternative and renewable sources of raw materials.

BACKGROUND

Biological diversity is the name we give to the occurrence of many of the different kinds of organisms found in the world. It is important to recognize that biological diversity is an outward evidence of chemical diversity. All organisms interact with other organisms and their environment by chemical means. Plants, organisms which are fixed in place and cannot flee injury, have evolved chemical defenses to protect themselves. Many insects find mates by releasing attractant chemicals into the environment to allure mates. Prey and predators interact through chemical scents as well as sight. Even humankind's exploitation of organisms is based largely upon our utilization of specific chemicals produced by those organisms. All of our foods are, in reality, chemicals as are our natural fabrics and all of our medicines.

Human survival has always depended on plants. Early humans relied entirely on them for food, medicine, much of the clothing and shelter. The botanical skills should not be underestimated. All of the world's major crops were brought in from the wild and domesticated in prehistoric times. An adequate food supply is, and always has been, humankind's most outstanding need. That food shortages are often due to political, socio-economic, unequal distribution or even cultural issues is recognized. However, in many cases, shortages are caused by attempts to employ inappropriate technology or to grow inappropriate crops.

At the present time, our most effective methodology for increasing crop productivity is through the utilization of chemical substances which reduce the effects of environmental and biological stresses upon the crop plants. Those chemical substances, pesticides and growth regulators, must have selective activity and be non-persistent in the environment. Agrochemicals developed from natural product prototypes will meet those stringent criteria.

Paralleling humankind's need for food is our need for agents to treat our ailments. The practice of medicine by physicians today in the United States and Western Europe is very different from the practice of their predecessors. This is largely because modern doctors have available a large array of medicines with specific curative effect. However, we lack specific curative agents for a number of important diseases. In the United States heart disease, cancer, viral diseases (for example AIDS), antibiotic resistant infections, and many others still lack adequate treatment.

Progress in the future towards discovery of cures of the serious diseases which still afflict humankind (cancer, viral infections, tropical diseases, etc.) and selective and environmentally benign

agrochemicals depends upon discovery and development of new chemicals with the desired biological activities. The search for such desirable new chemicals takes two general forms: (1) preparation by synthesis in the laboratory and (2) examination of the substances present in living organisms, called natural products. This latter approach derives from the historical recognition and use of plants and microorganisms in various forms in medicine and agriculture; the systems of traditional practices upon which two thirds of humankind still rely for their medications. Natural product research has been conducted for nearly 200 years. It formed the basis for the discovery and development of a majority of the pharmaceuticals in use today. In 1990, world-wide, nearly 4000 new chemical structures were characterized, of which roughly three-quarters were isolated from plants. As much as 10% of the dry mass of some plants may be made up of chemicals for defense against predators or infection. Evolution of plants has proceeded in many directions, leading to creation of uncounted chemical defense substances, only a few of which have been identified. Fewer of them have been evaluated for their potential usefulness as drugs or agrochemicals. Natural products represent a proven yet largely unexploited source for the discovery and development of new chemotherapeutic agents and agrochemicals.

The National Center for the Development of Natural Products has established an integrated and multi-disciplinary approach to discover, develop and commercialize new pharmaceutical and agrochemical products from natural sources. This process will begin with evaluation of plant derived natural products for potential as pharmaceuticals and agrochemicals, carry through to development and commercialization of products derived from natural sources and finally, come full-circle by developing the plant sources of these products into alternative high value crops for farmers.

CONSORTIUM CONCEPT

The realization of the potential of natural products as new chemotherapeutic agents and agrochemicals is dependent upon harnessing diverse activities for discovery, development and commercialization of these substances. Only by formation of a university/federal/state/private sector partnership can we be assured that sufficient strength in all the requisite activities, especially commercialization, will be available to develop new drugs and agrochemicals from natural products. A **consortium** of the Research Institute of Pharmaceutical Sciences, the Agricultural Research Service and Cooperative State Research Service of the USDA and private companies is the most effective mechanism to accomplish this goal.

NATURAL PRODUCTS: THE PERSPECTIVE OF THE AG CHEMICAL INDUSTRY

Presented by
Carl E. Snipes
DowElanco
P.O. Box 68955
Indianapolis, IN

The discovery and optimization of new leads for crop protection is essential to the future of the Ag Chemical industry, and natural products are a proven source of new chemistry with diverse, interesting, and commercially exploitable activity. The costs of product development, registration, manufacturing, and marketing dictate that resources must be focused on product concepts that address major global markets in ways that allow the value of delivering reliable protection to be captured. The value of the commodity crops in production and the limited ways of delivering the crop protection to the pest limit our ability to overcome all obstacles in optimizing the performance of a lead, even when the intrinsic activity is very high. The elements of a successful natural products screening program include definition of the product concept, how to screen for the activity, how to measure "commercial performance" potential at an early stage, and how to eliminate known compounds at an early stage. The sources of input into the screen can include high throughput random sources and lower sample number but more intensely studied targeted sources and lower sample number but more intensely studied targeted sources such at pathogens and resistant plants. Alternatives to traditional chemical control strategies continue to have a high level of interest, but most of these have limitations that prevent them from becoming significant commercial opportunities. In summary, natural products have the potential to have a large impact on the future of crop protection, and the ARS needs to play a role in this important area of research.

REGULATORY CONSIDERATIONS FOR TECHNOLOGY TRANSFER OF NATURAL PRODUCTS

Presented by
Richard M. Parry, Jr.
Deputy Assistant Administrator
Office of Technology Transfer

The Administration of the Federal Government has set the goal for implementation of biologically-based Integrated Pest Management (IPM) programs on 75 percent of the nation's acreage by the end of the century. ARS will be aggressively moving to realize this

goal by providing technology for IPM systems in agriculture. Natural Products can be expected to play an important role in realizing this objective. Transferring this technology into commercial products is a particular challenge since many, if not all, the natural products that will be discussed fit under the legal definition of pesticides given in FIFRA. Information will be presented on the special constraints that may limit development of new pest control technologies which need to be considered in product development.

THE IR-4 PROGRAM

Presented by
N. P. Thompson
University of Florida
Gainesville, Florida

IR-4 is making an increasing commitment to the registration of biorational pesticides which have been part of the program objectives since 1983. Research supported emphasizes those strategies which have reasonable possibilities for short term transfer from research to the marketplace. Guidelines have been promulgated which govern the nature of the research supported and the review process. Projects must be ones subject to registration under FIFRA Subpart M, have a definite need and a reasonably high potential for industrial production and generate enough economic incentive to have the material produced and marketed as a biorational. Research can include provision for Tier I studies when necessary.

THE ARS PATENT PROCESS

Presented by
Gail E. Poulos
USDA, ARS
Office of Technology Transfer
Bldg. 005, BARC-West
Beltsville, MD 20705

ARS scientists need to be aware of patent possibilities early in their research to provide time to consider what type of protection, if any, should be pursued by ARS. It is important to communicate with a patent advisor in the Office of Technology Transfer soon after the scientist realizes that the research may produce an invention that ARS should consider patenting. The scientist and the patent advisor can then work together to preserve the option of obtaining both U.S. and foreign patent rights without delaying publication or public oral disclosure of the scientist's research. In some technologies, it is important

to obtain both U.S. and foreign patent rights in order to license the invention. Most foreign countries have a absolute novelty requirement which means that any public disclosure, oral or written, will compromise any foreign patent rights. In the U.S., we have one year to file an application in the U.S. Patent and Trademark Office (PTO) after public disclosure. Some areas that may be deemed as publications by the PTO if the invention is fully disclosed are (a) CRIS Reports, (b), Technical and Interpretive Summaries on the Request to Submit Manuscript for Publication, and (c) abstracts for talks or poster sessions at conferences. However, to date, CRIS Reports have not compromised U.S. or Foreign Patent Rights. The technical and interpretive summaries are publicly available on the ARS database TEKTRAN unless the question, "Due to patent potential, is retention of intellectual property rights desired?", is answered "yes". So in this case, ARS has some control over this disclosure. Once a patent application is filed in the United States, ARS has one year to determine if it will be necessary to obtain foreign patent rights in countries that have signed the Patent Cooperation Treaty (PCT). For those countries that are not a part of this treaty, applications must be filed before the U.S. patent issues and/or before any public disclosure occurs. A PCT International application can be filed designating countries where protection is desired. This would give ARS at least 18 months to determine if it would be in the Agency's best interest to pursue both U.S. and foreign patenting. After 18 months the PCT application is published and made available to the public. If patent rights are pursued, the agency has up to 30 months to determine which of the designated countries are worth pursuing at the National stage of patenting. Generally, a license is obtained on the technology before the 30 month period has elapsed and the licensee could provide ARS guidance on which of the designated countries would be the best for obtaining patent rights and reimburse ARS for the costs. As can be seen from the above, early communication with the Office of Technology Transfer helps in making decisions for appropriately transferring technology to the public.

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